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(54) **FUNGUS-INDUCED INFLAMMATION AND EOSINOPHIL DEGRANULATION**

(75) Inventors: **Hirohito Kita**, Rochester, MN (US);
Jens Ponikau, Amherst, NY (US);
Christopher Lawrence, Blacksburg, VA (US)

(73) Assignees: **Mayo Foundation for Medical Education and Research**, Rochester, MN (US); **Virginia Tech Intellectual Properties, Inc.**, Blacksburg, VA (US)

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435/183; 530/350

(58) **Field of Classification Search** None
See application file for complete search history.

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Primary Examiner — Jennifer E Graser

(74) *Attorney, Agent, or Firm* — Fish & Richardson P.C.

(57) **ABSTRACT**

This document relates to methods and materials involved in fungus-induced inflammation and eosinophil degranulation. For example, isolated nucleic acids encoding fungal polypeptides, fungal polypeptides, methods for assessing fungus-induced inflammation, methods for assessing eosinophil degranulation, and methods for identifying inhibitors of fungus-induced inflammation and/or eosinophil degranulation are provided.

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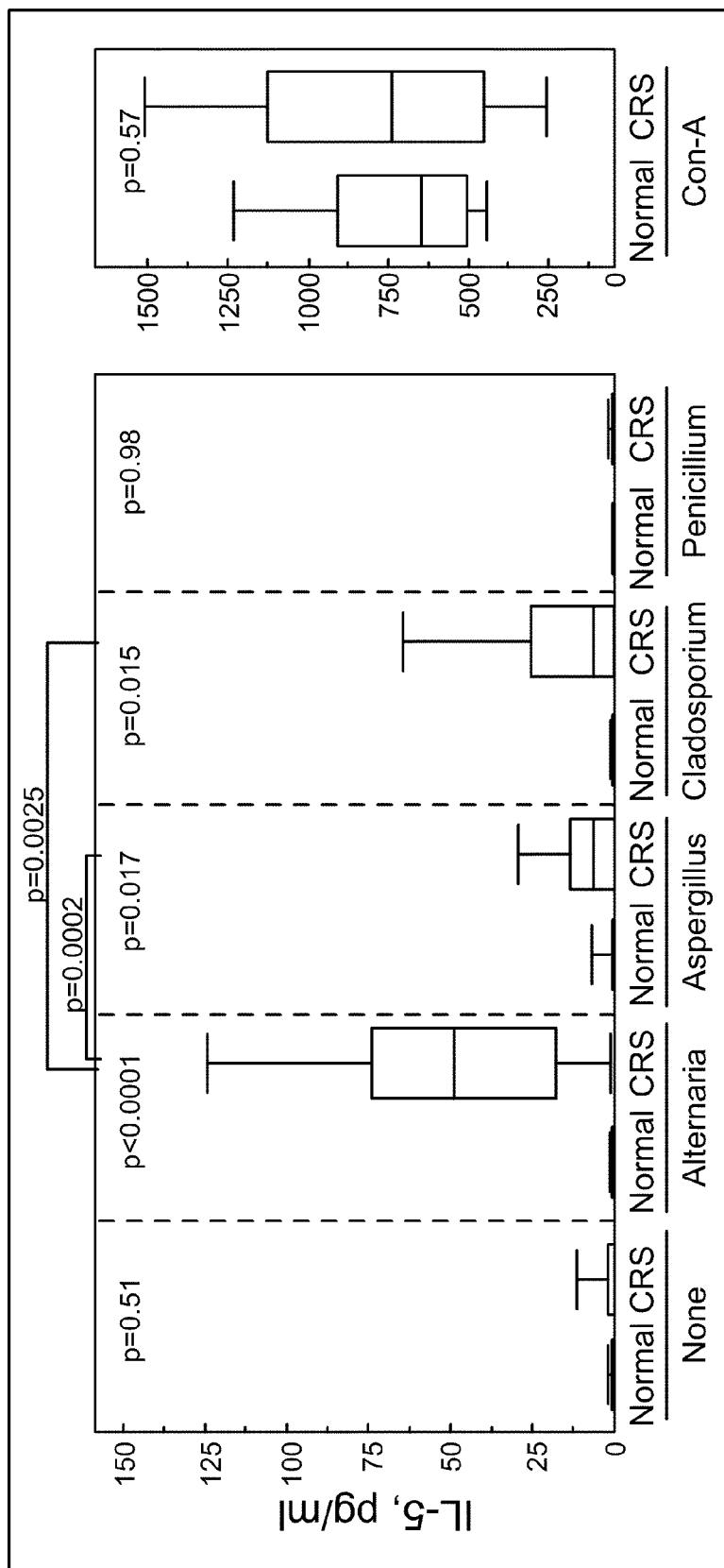


FIG. 1

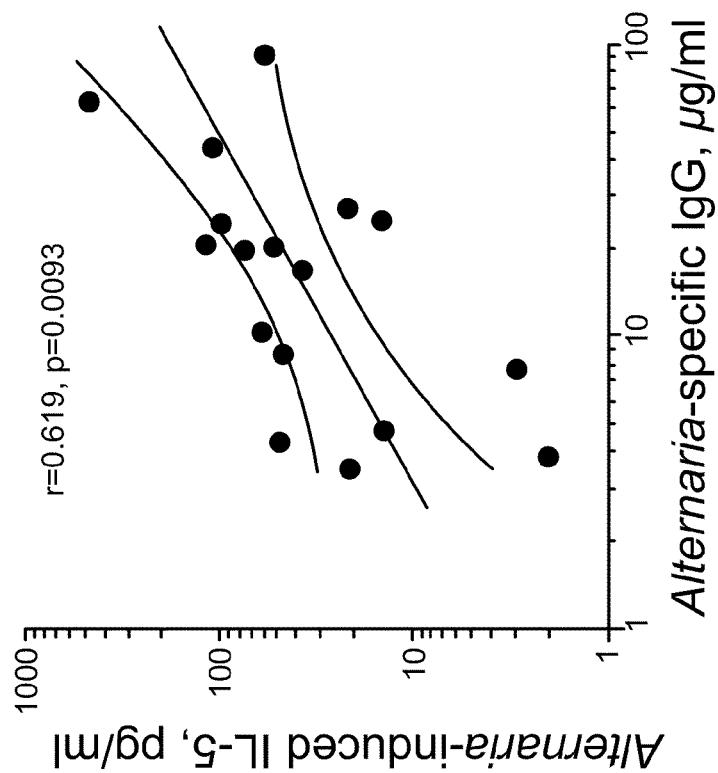


FIG. 2B

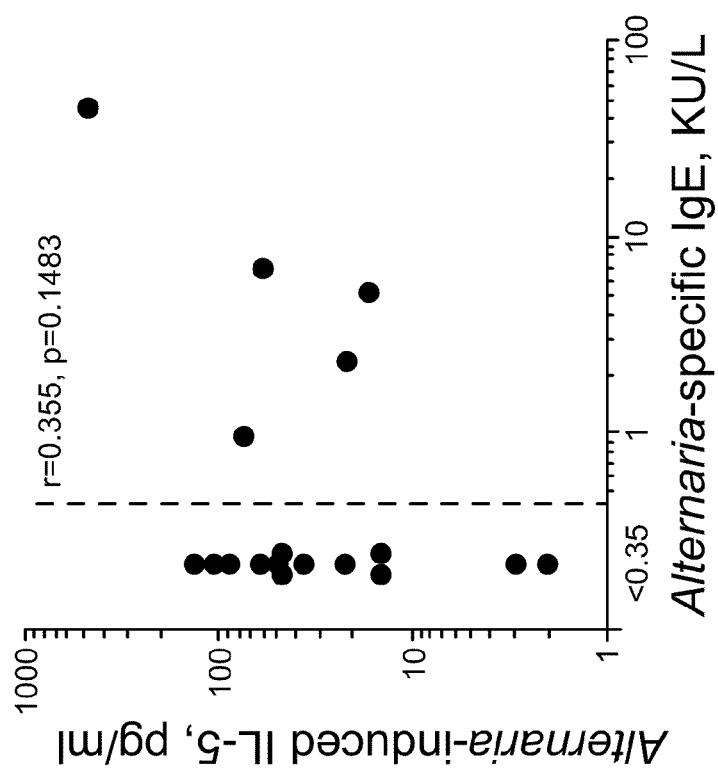


FIG. 2A

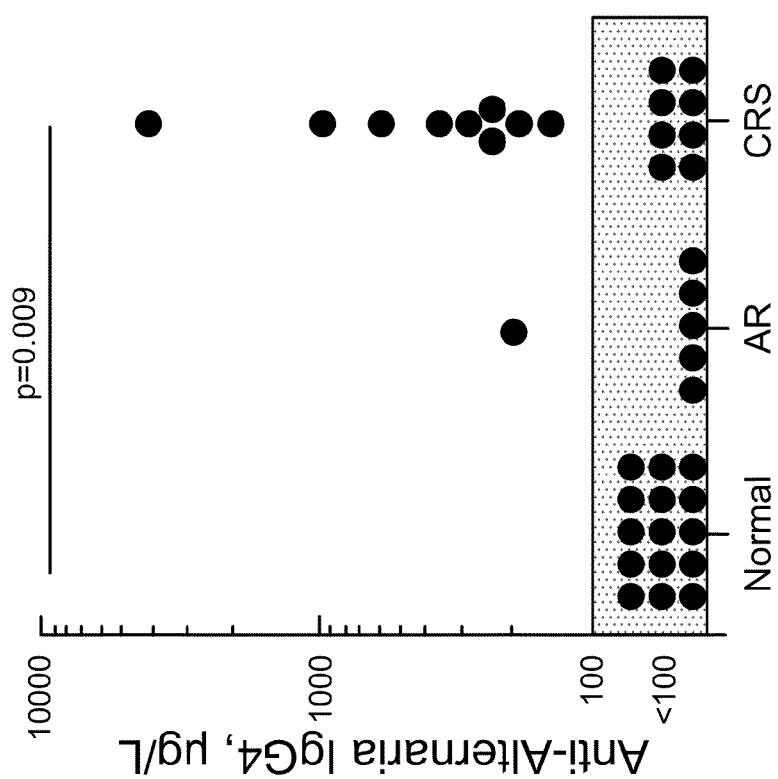
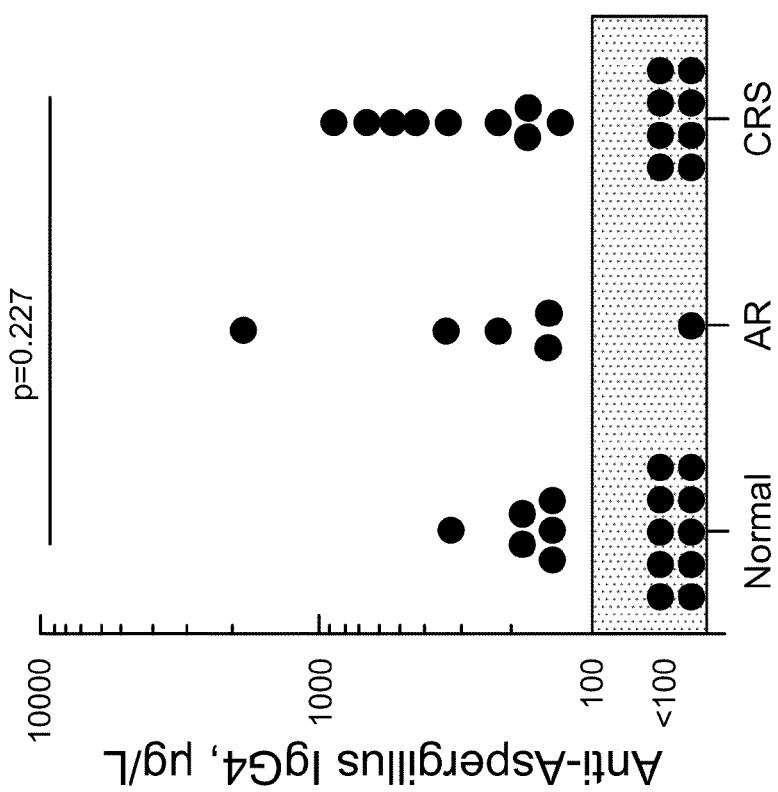


FIG. 3

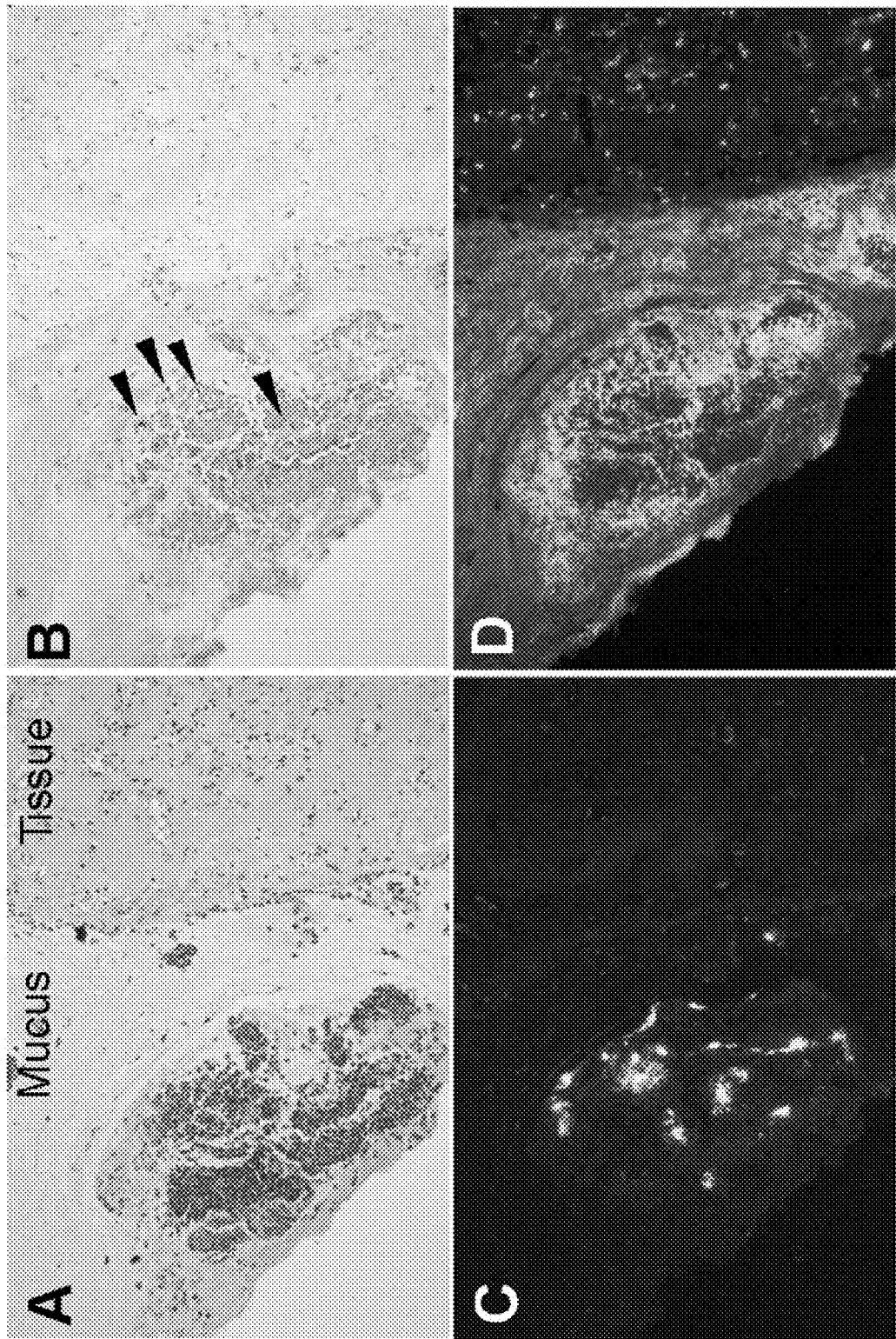


FIG. 4

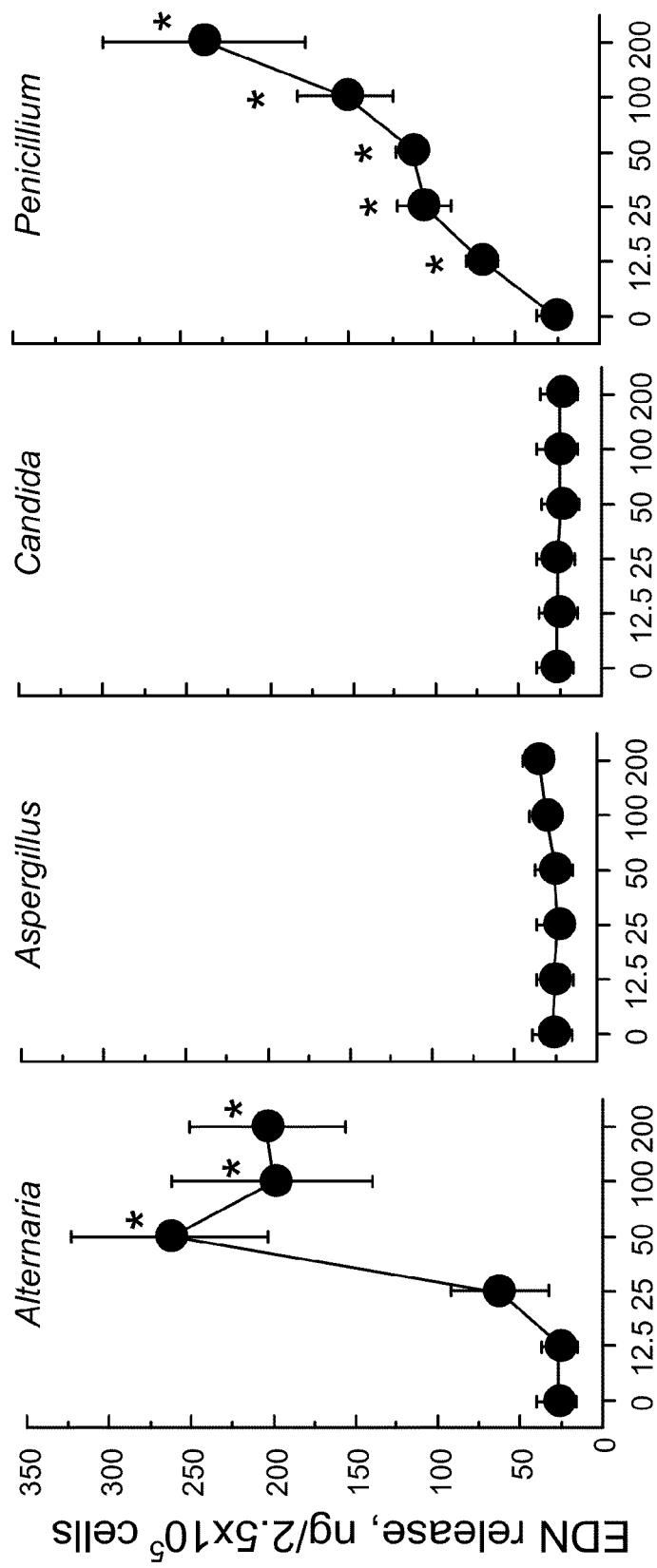


FIG. 5

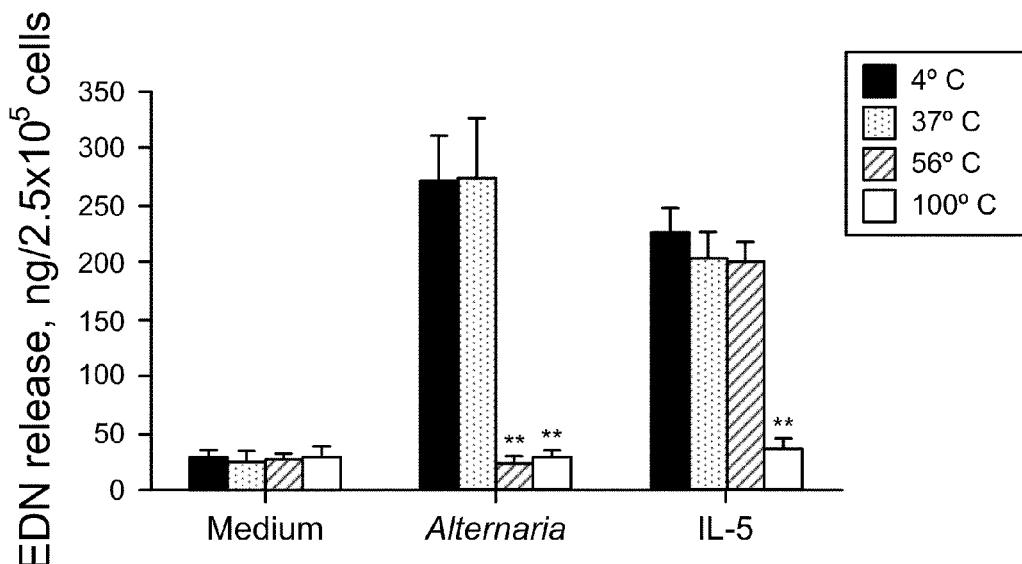


FIG. 6A

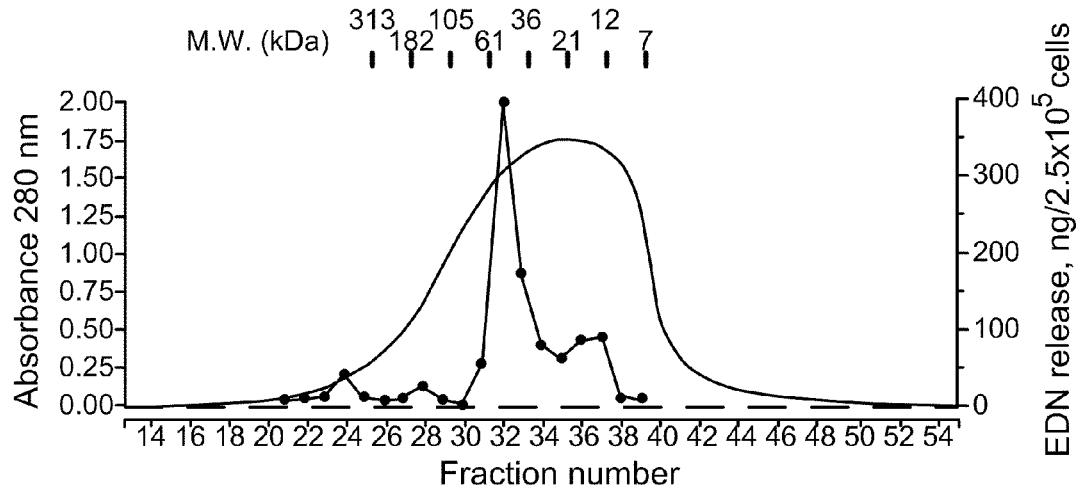


FIG. 6B

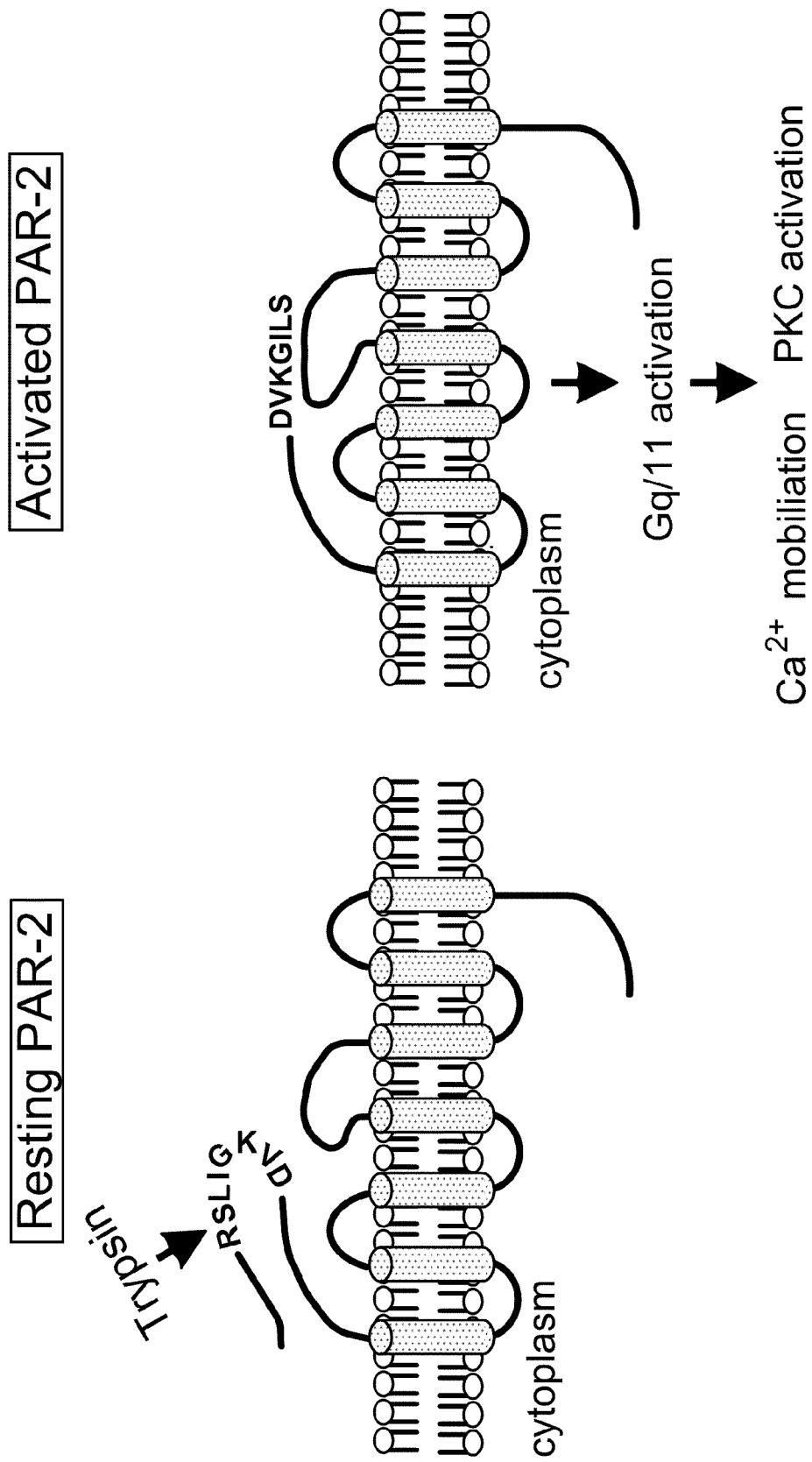


FIG. 7

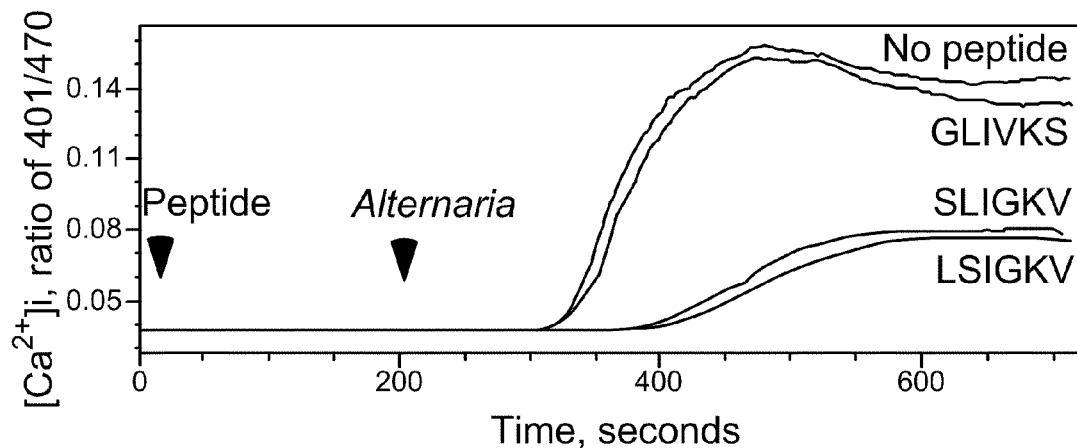


FIG. 8A

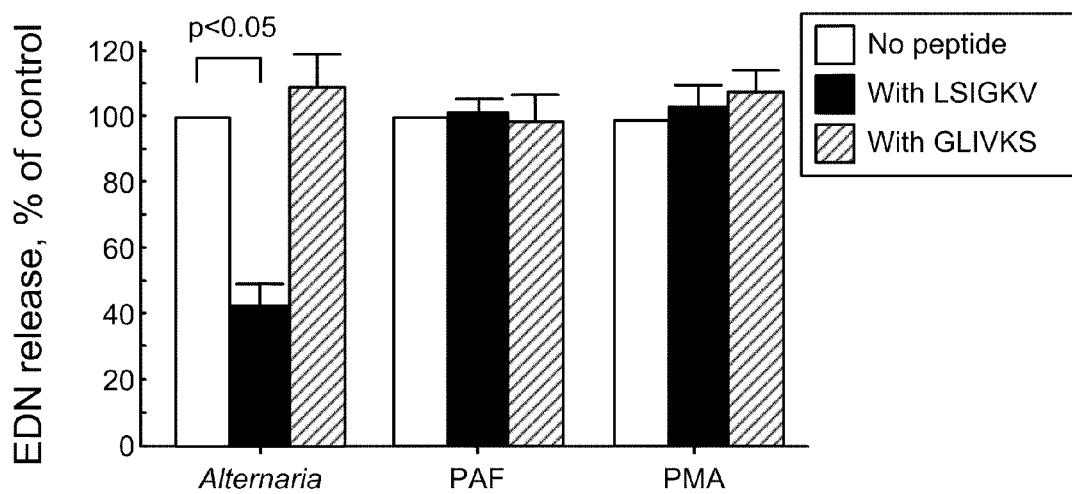


FIG. 8B

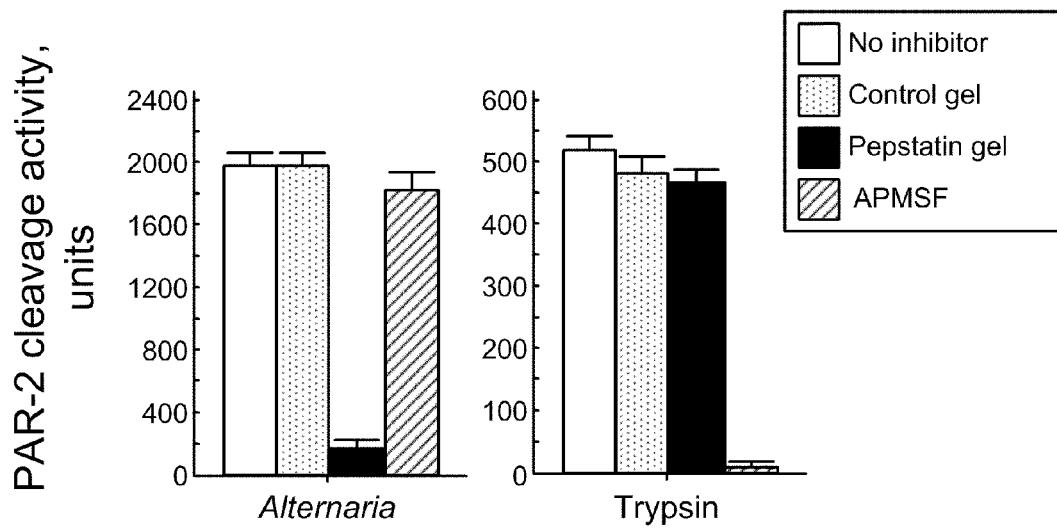


FIG. 9A

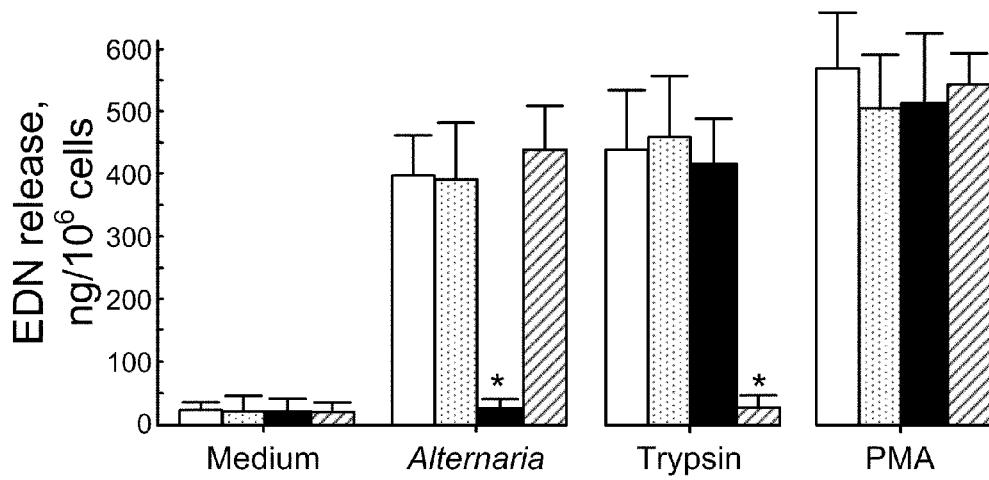


FIG. 9B

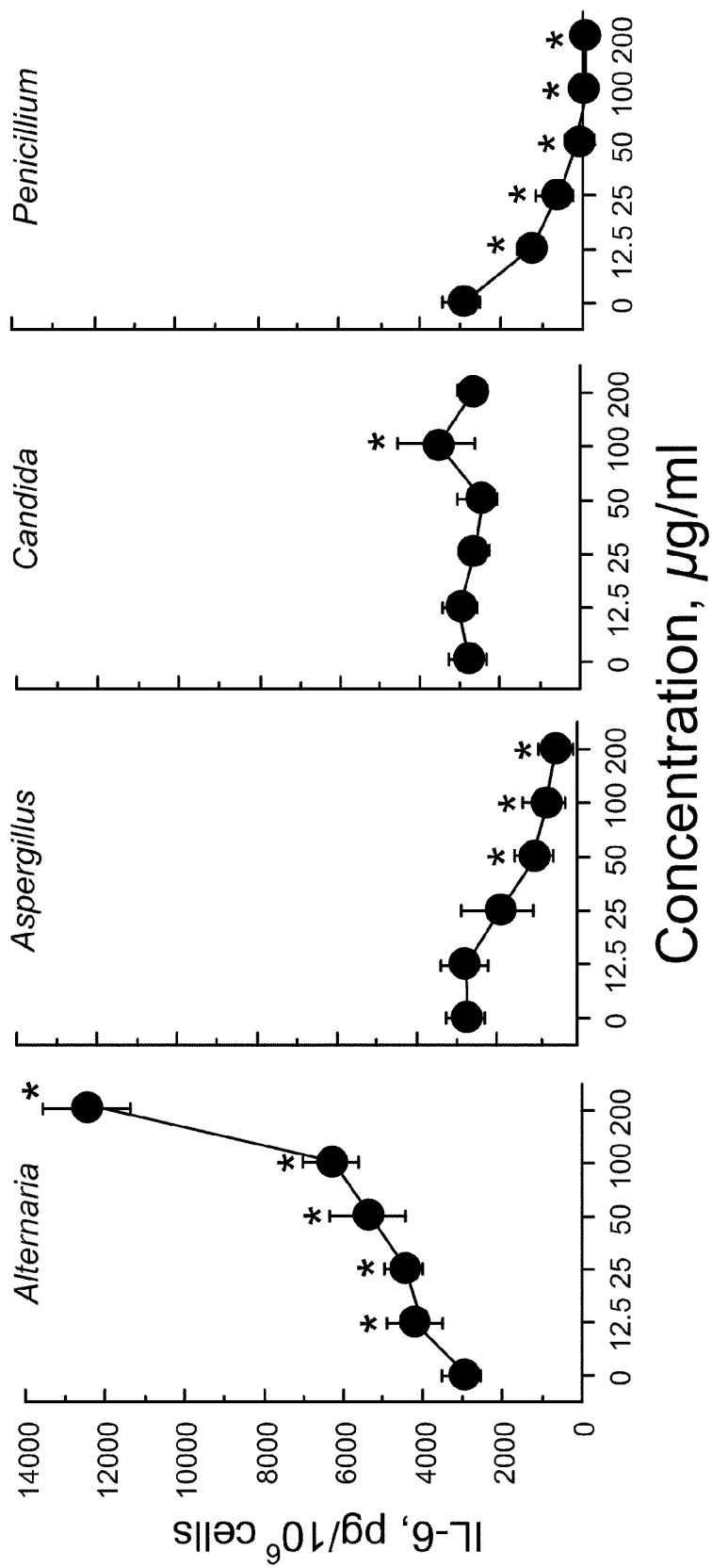


FIG. 10

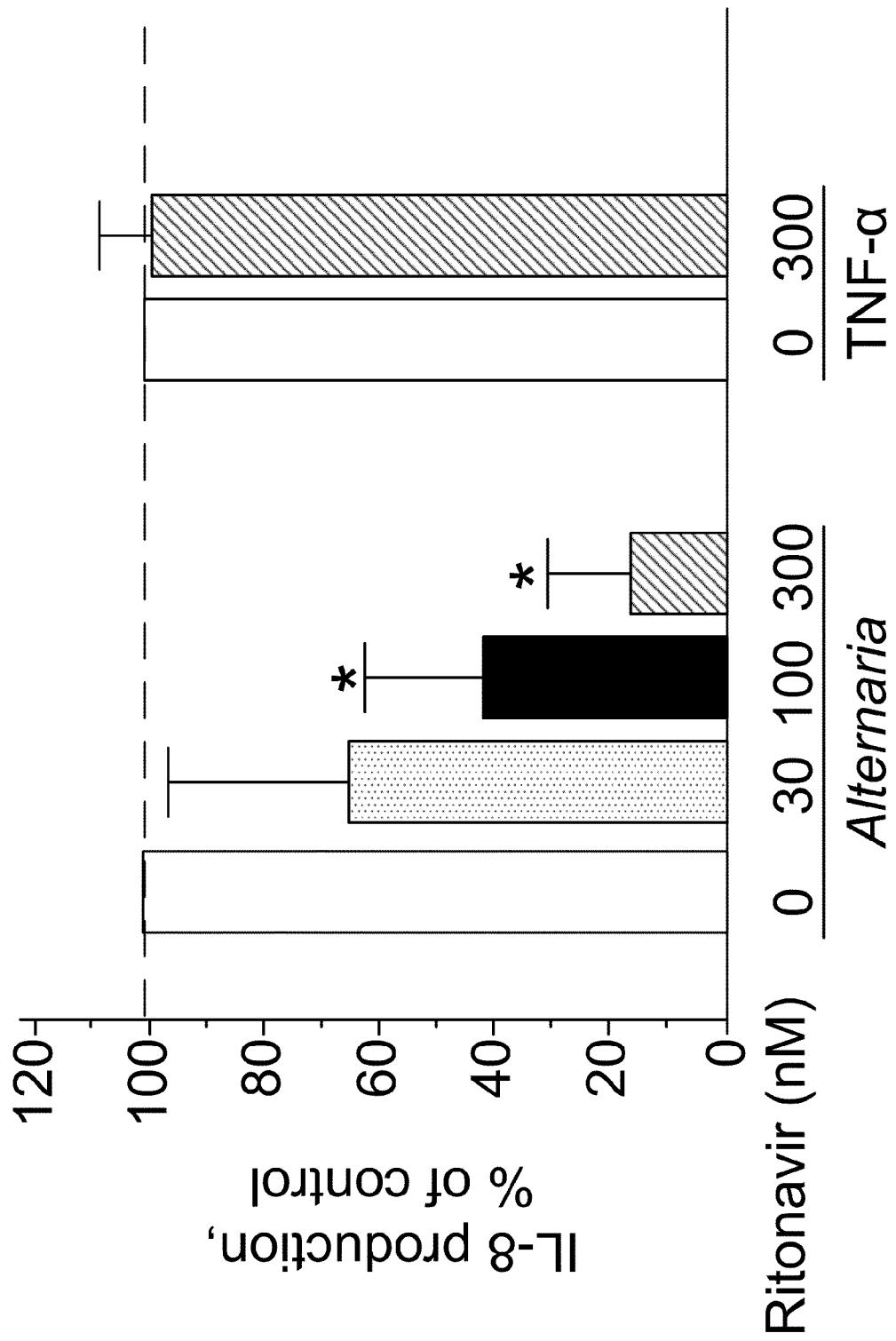


FIG. 11

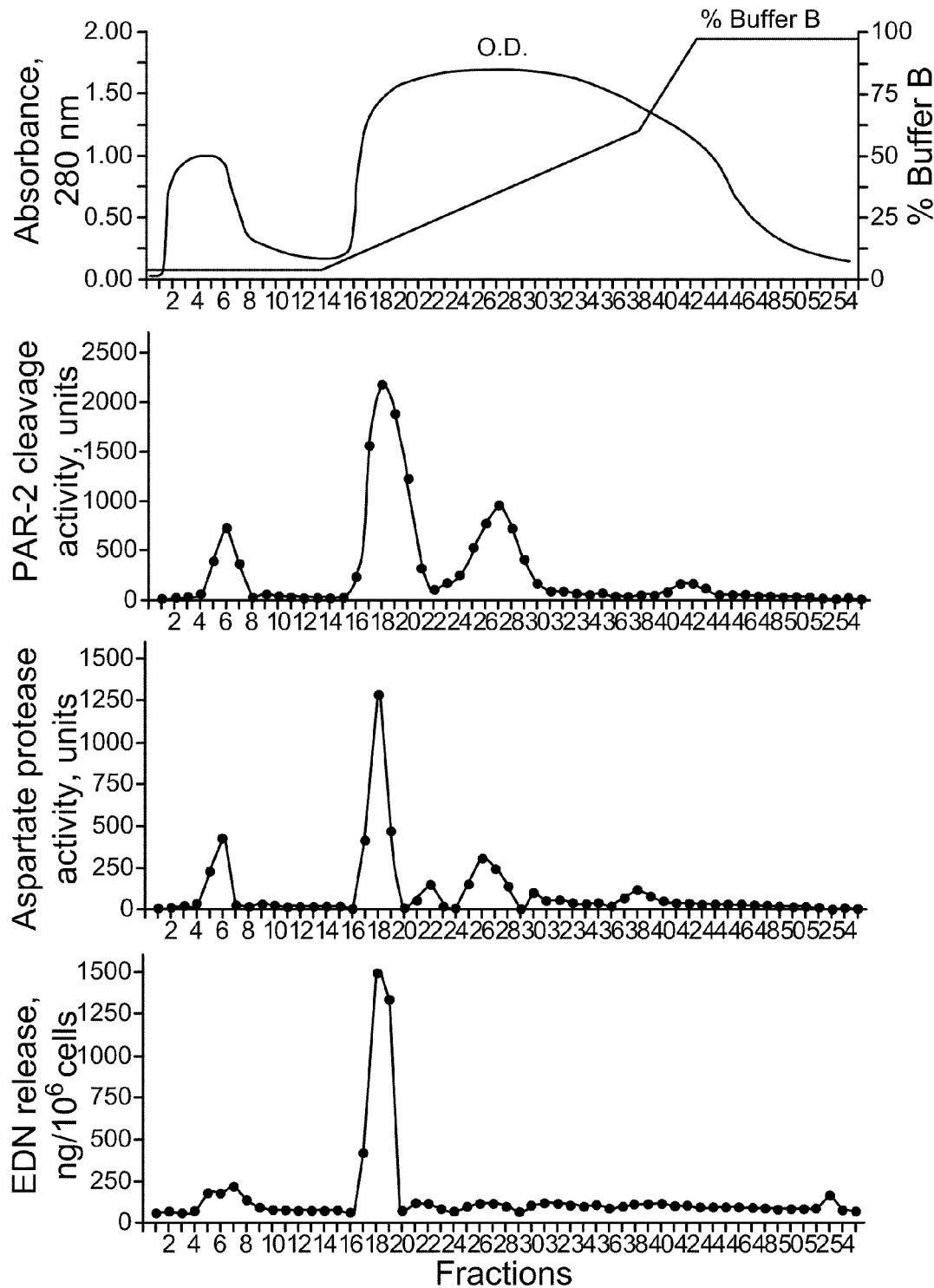


FIG. 12A

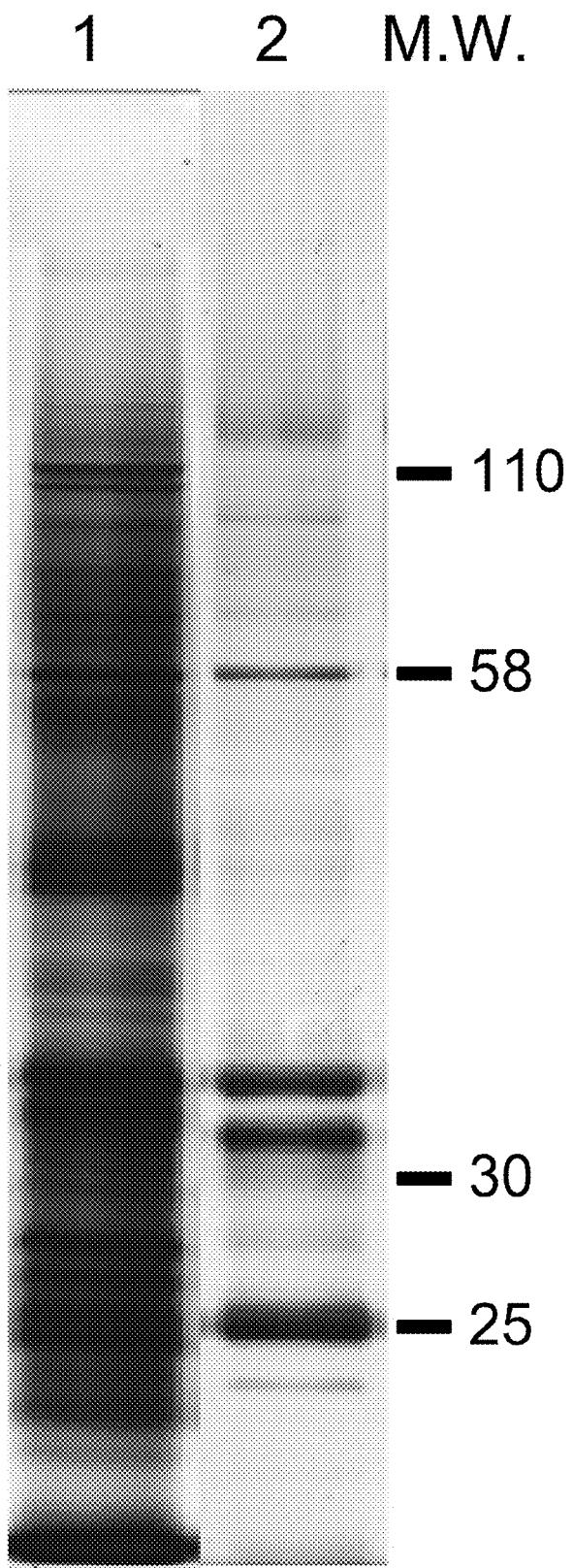


FIG. 12B

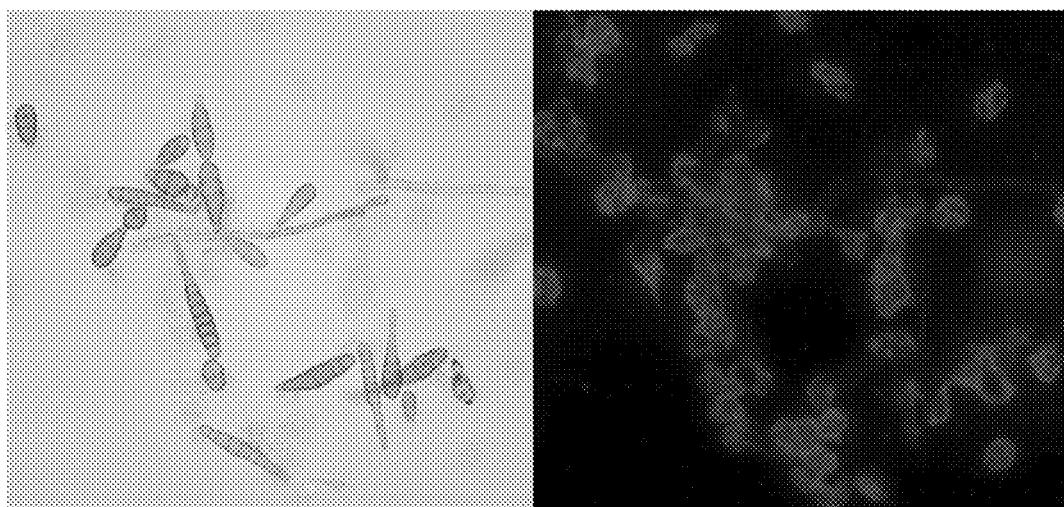


FIG. 13A

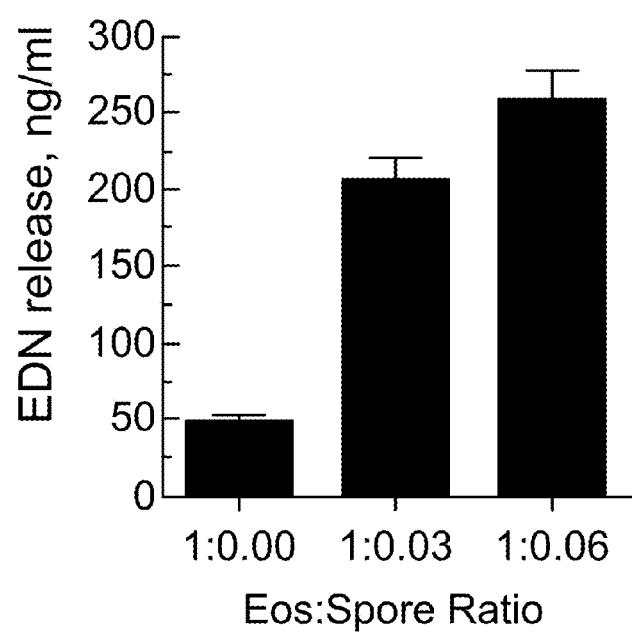


FIG. 13B

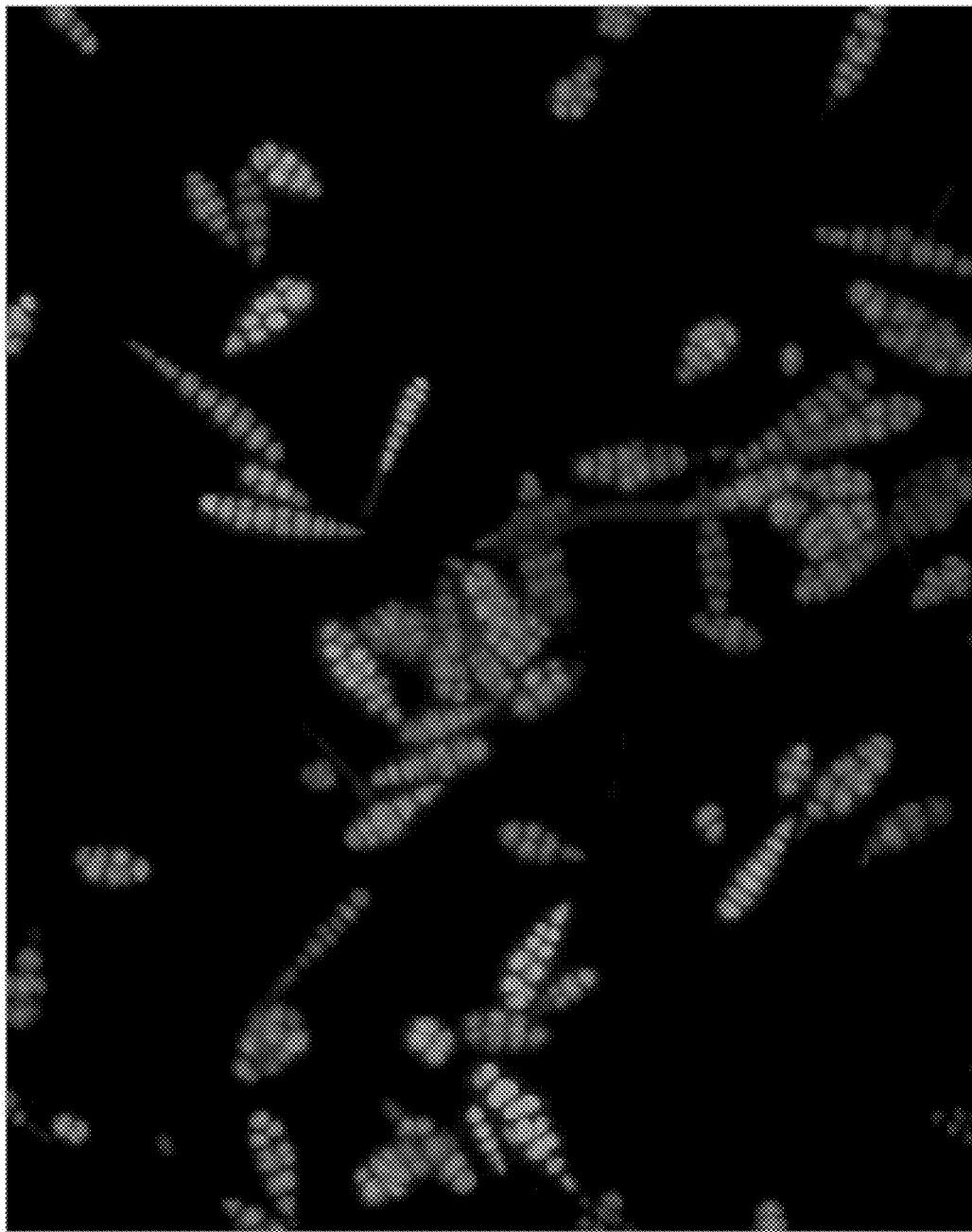


FIG. 14

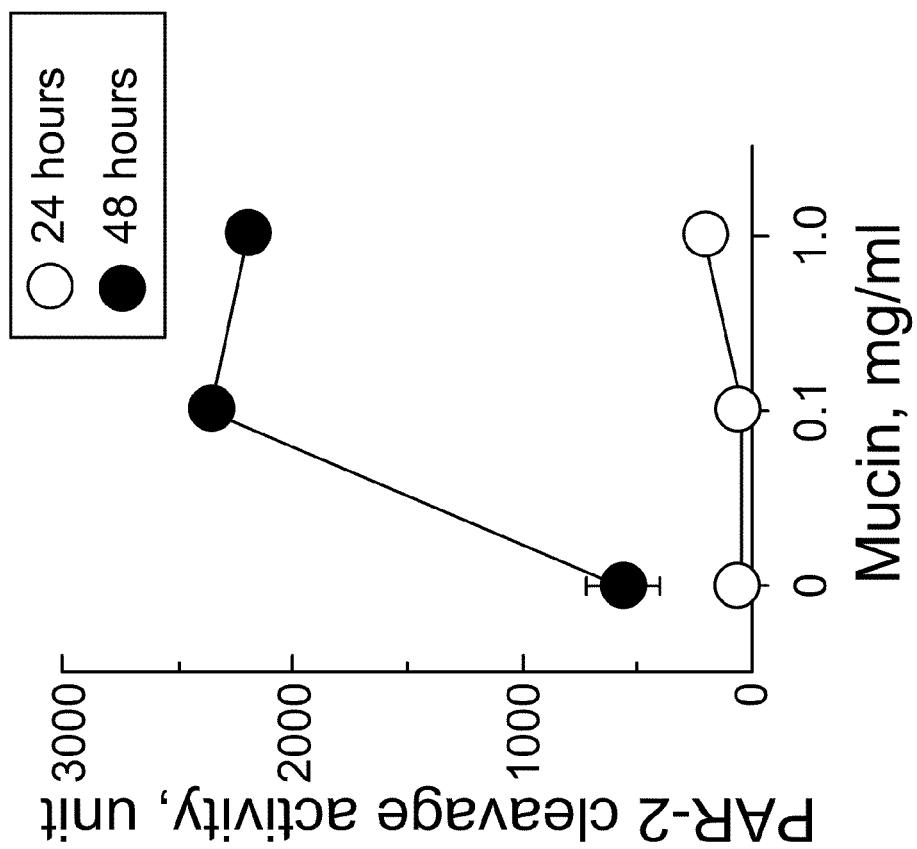


FIG. 15B

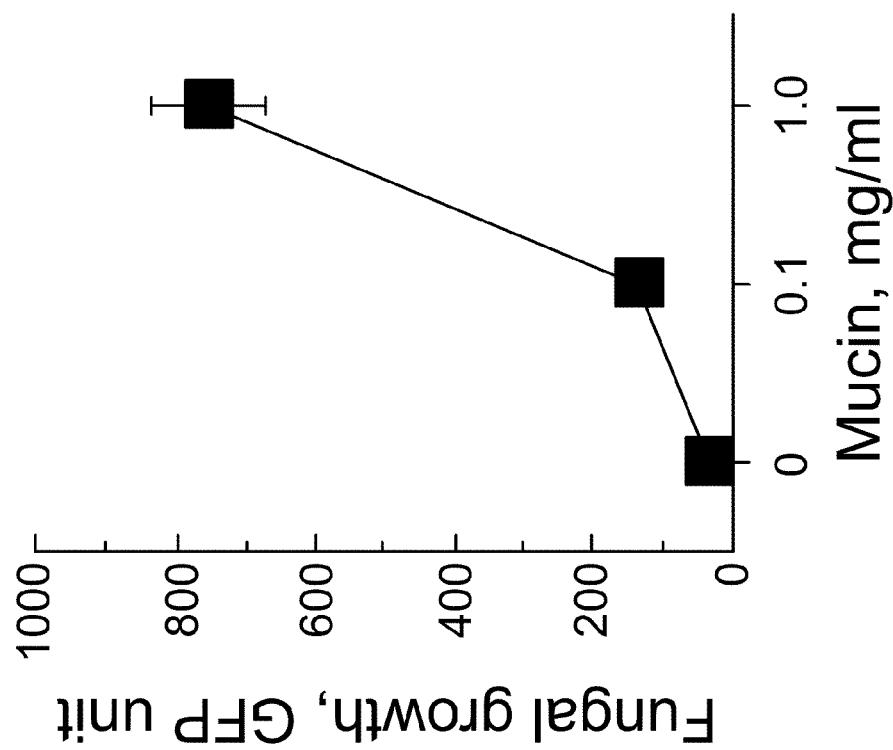


FIG. 15A

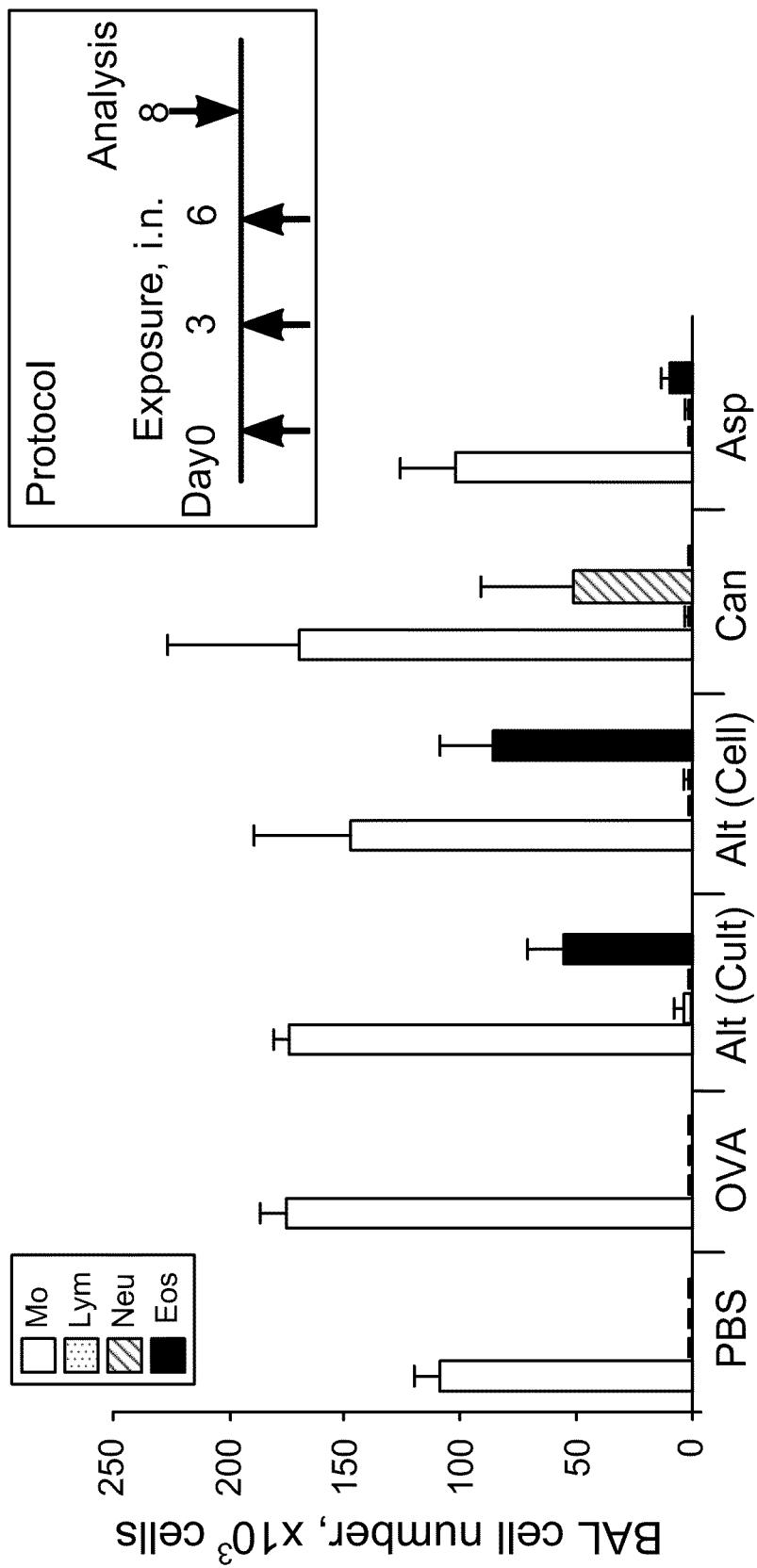


FIG. 16

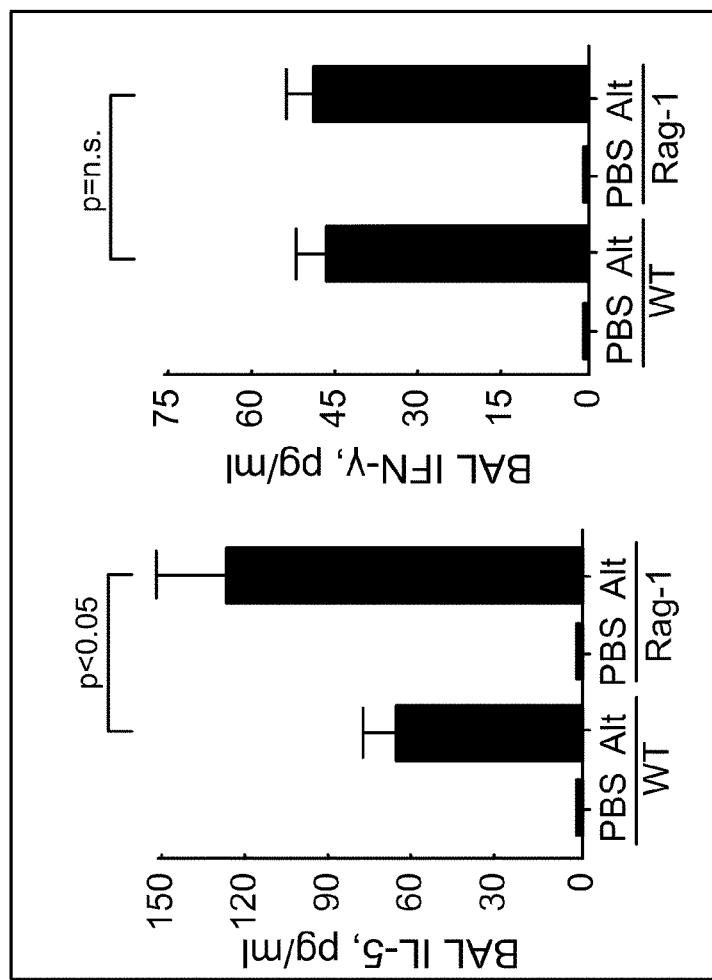


FIG. 17B

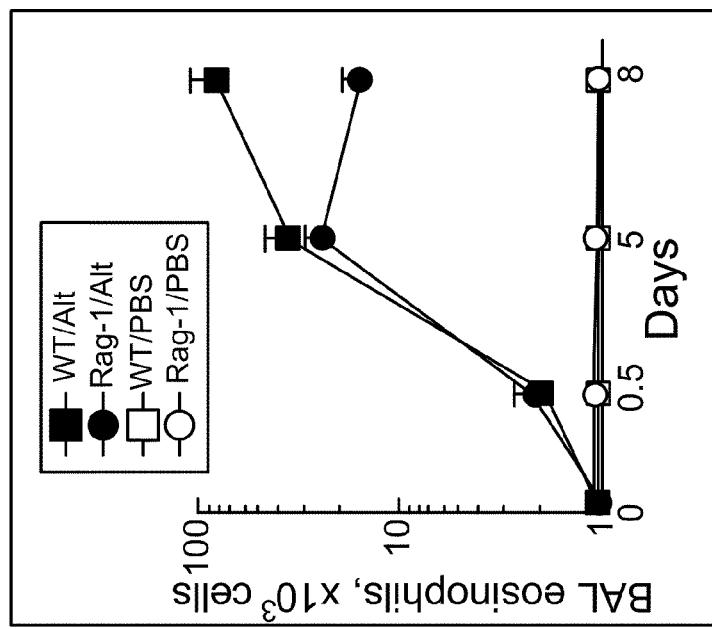


FIG. 17A

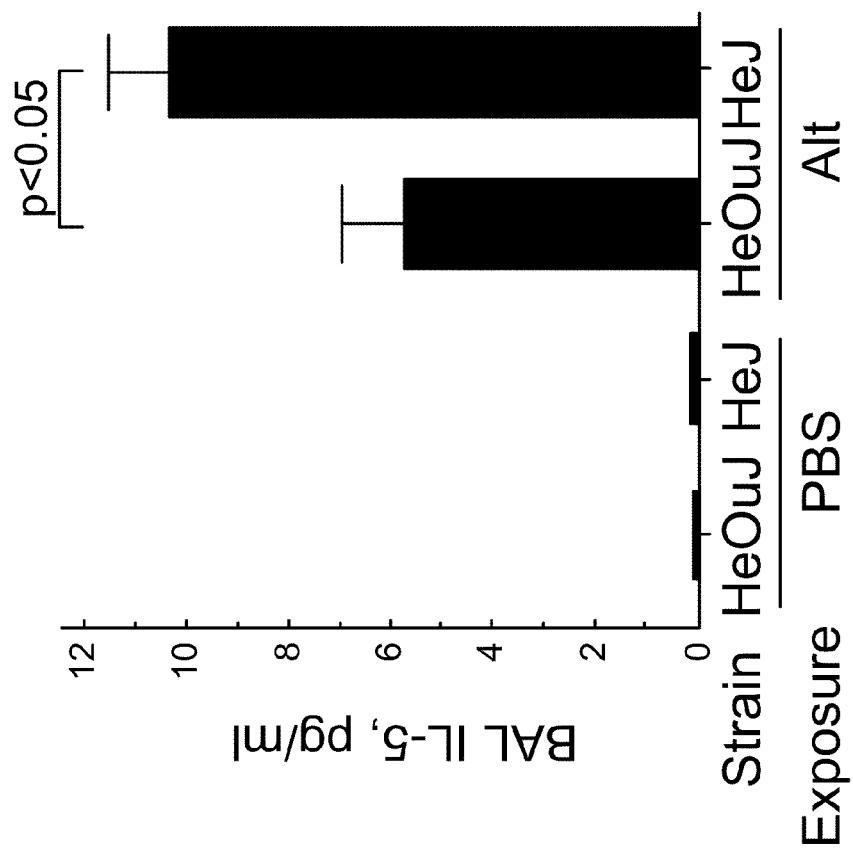


FIG. 18B

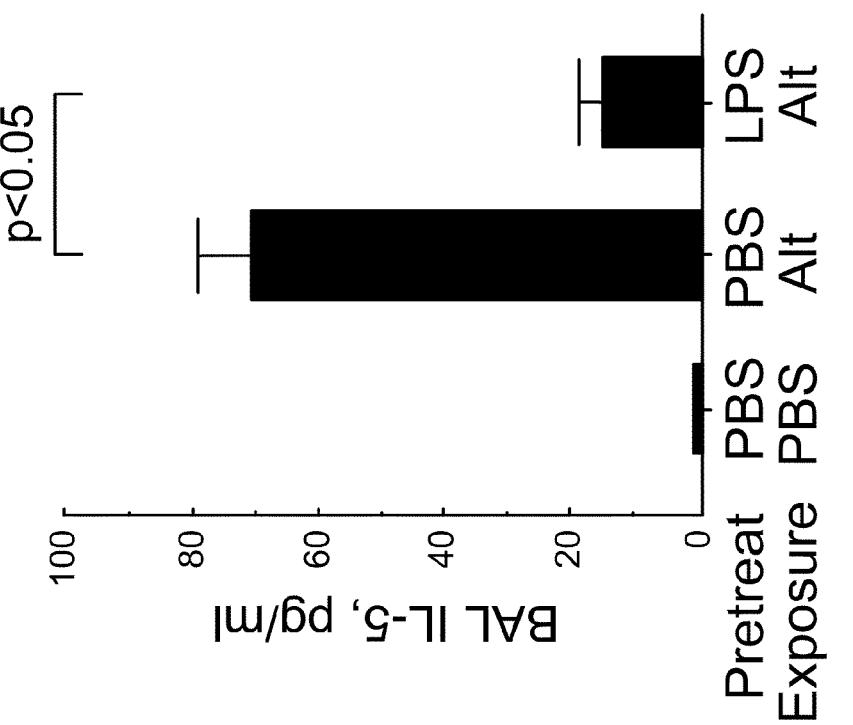


FIG. 18A

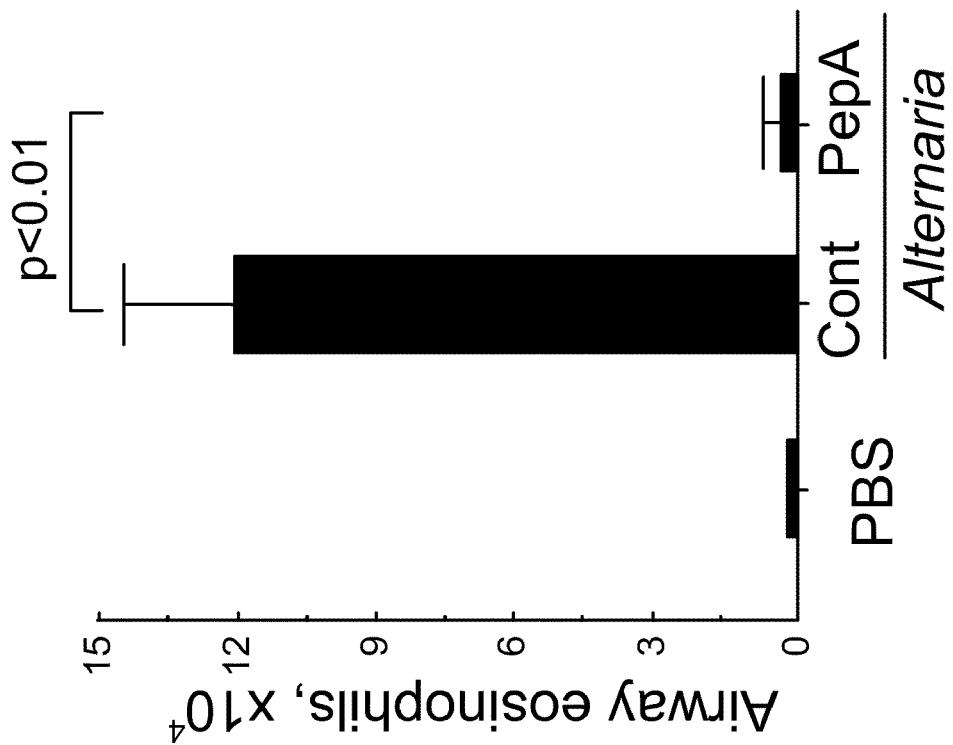


FIG. 19B

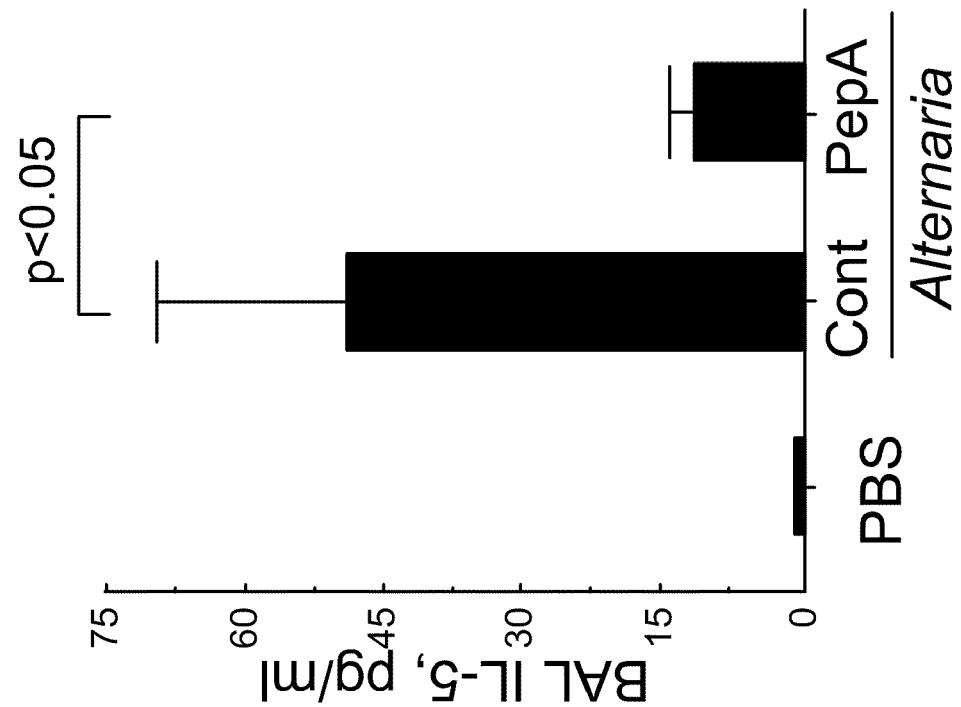


FIG. 19A

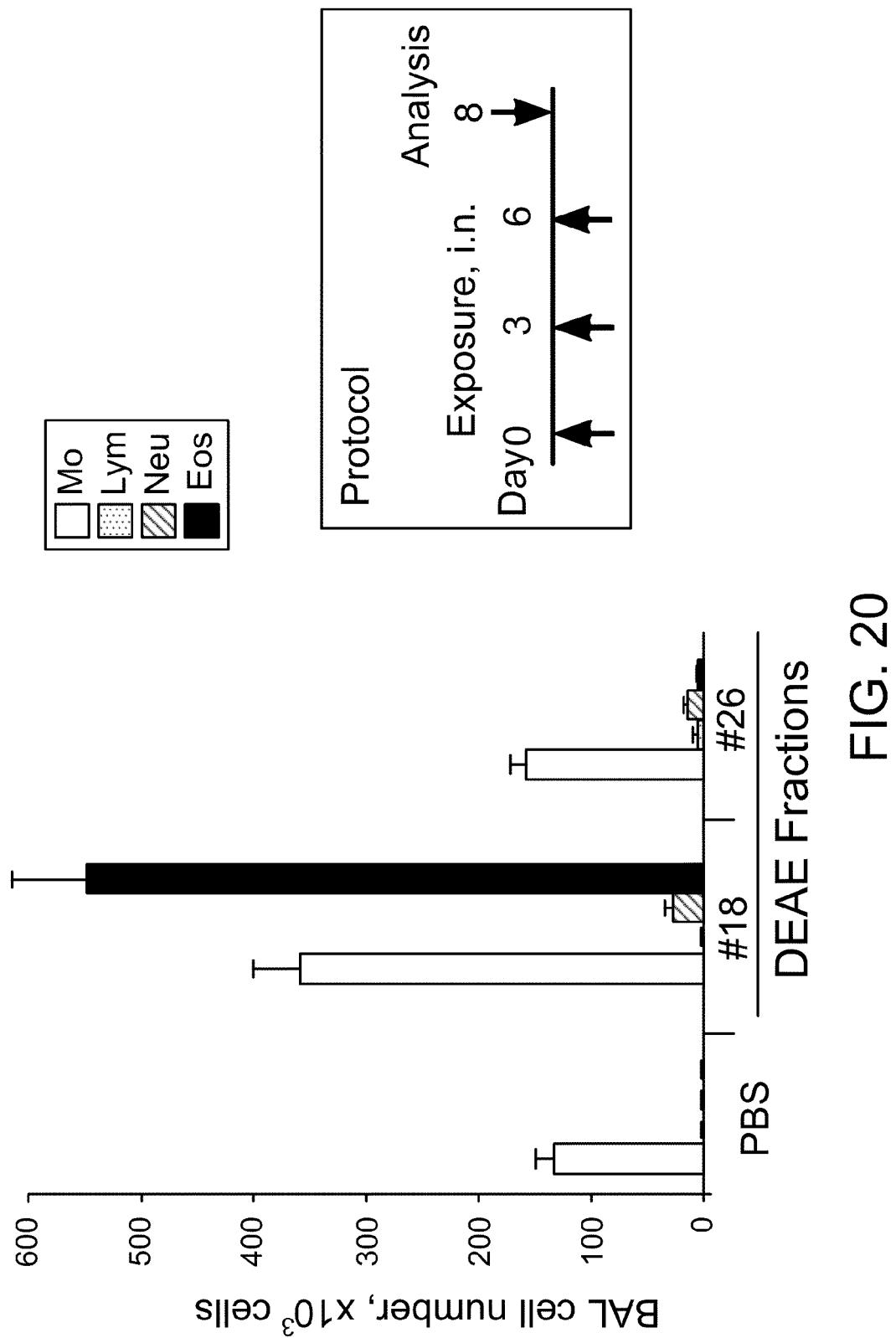


FIG. 20

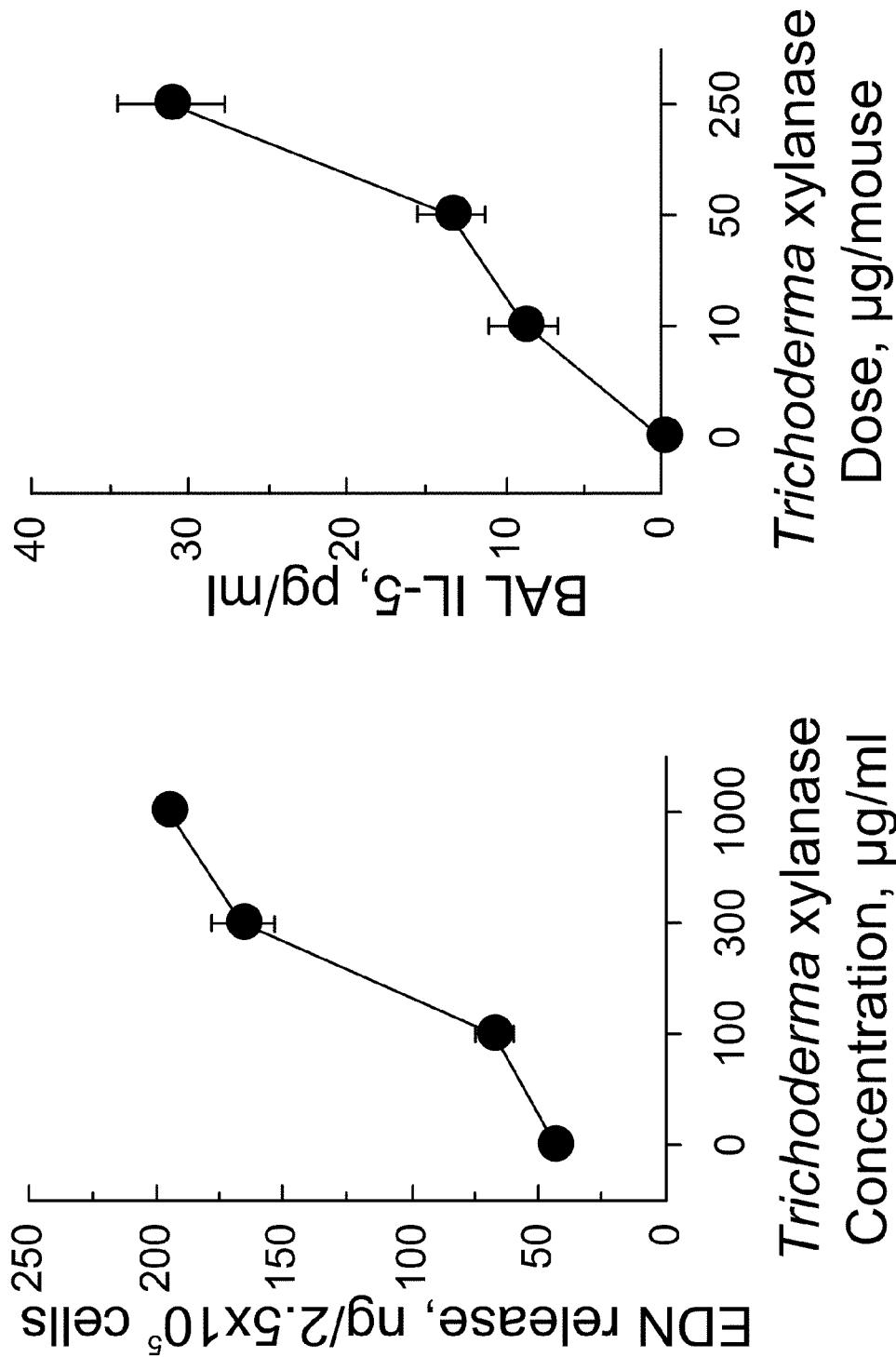


FIG. 21A

FIG. 21B

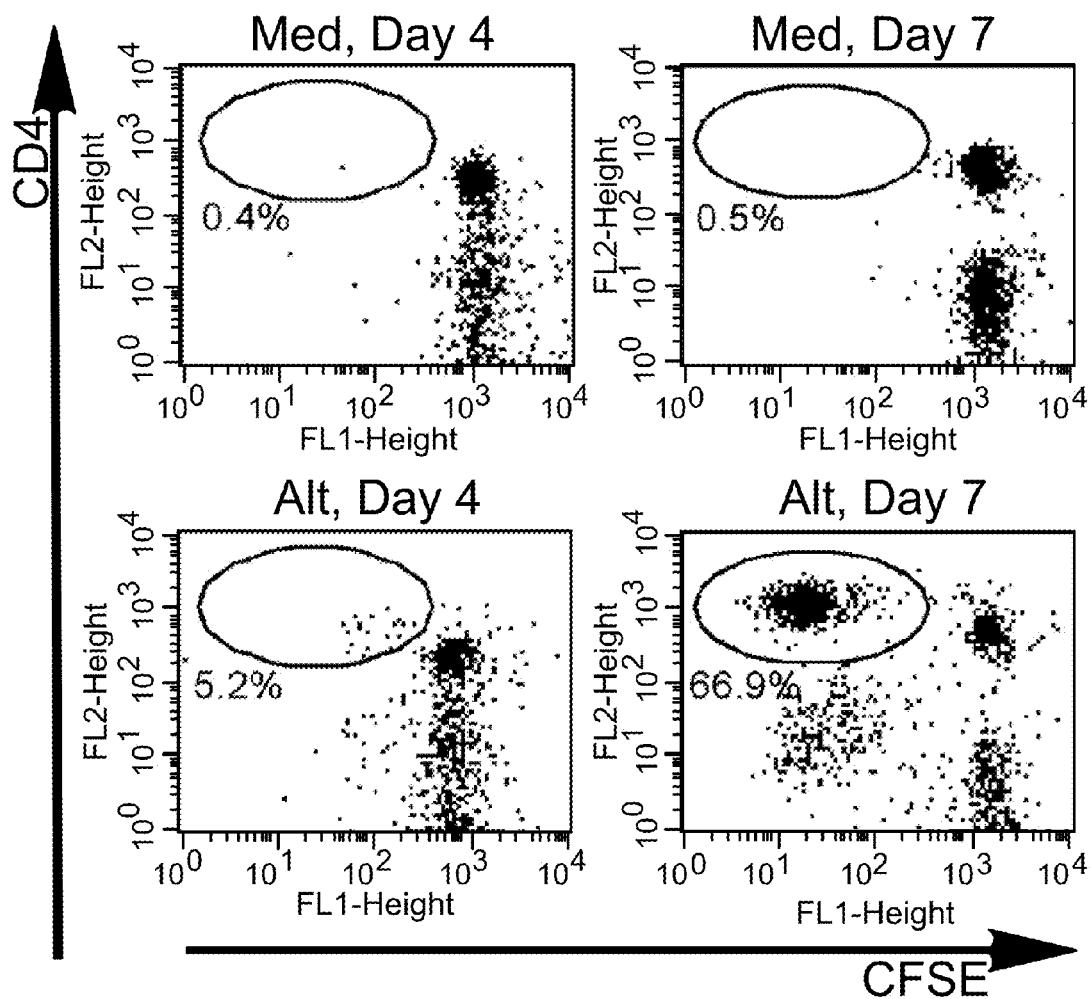


FIG. 22

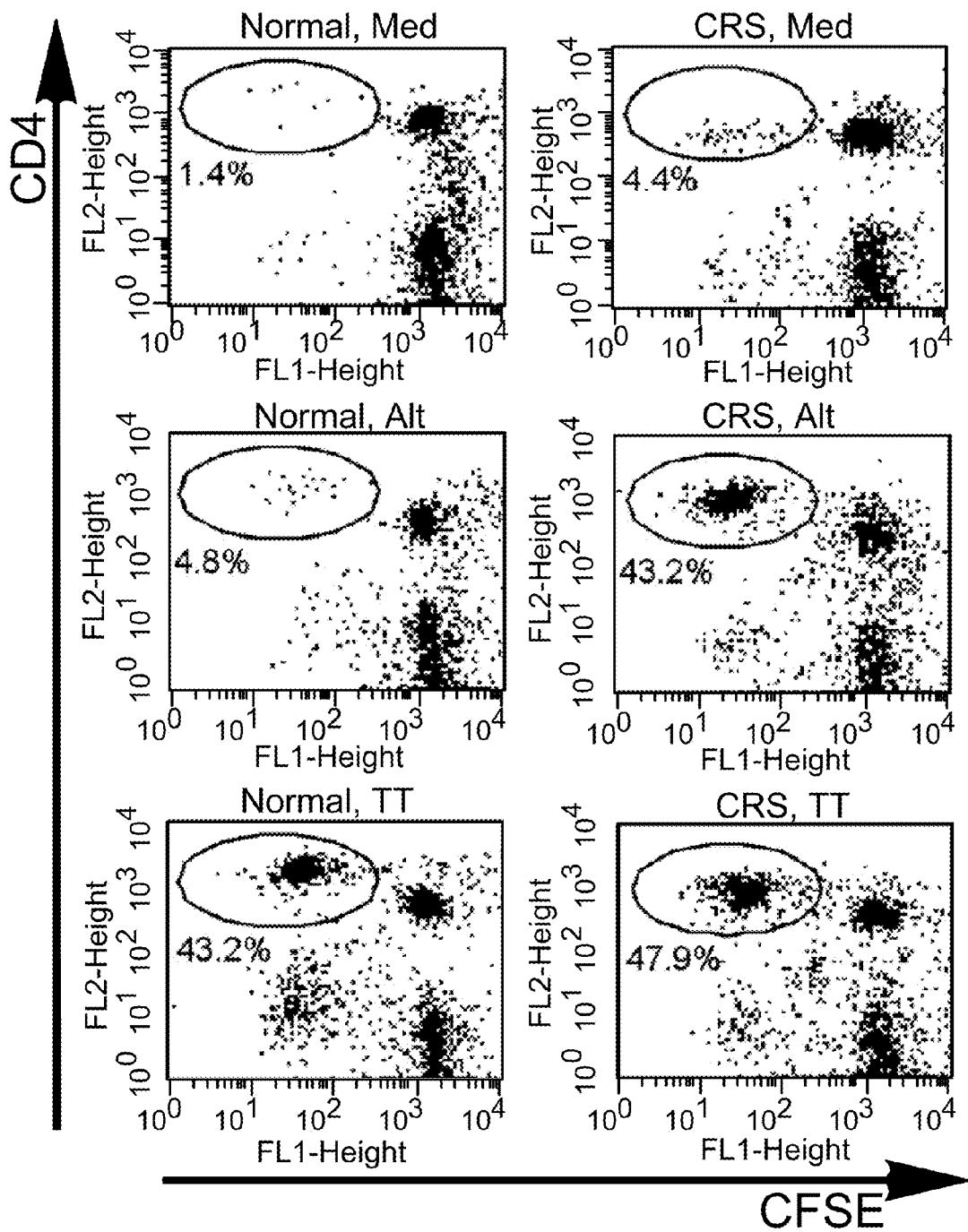


FIG. 23

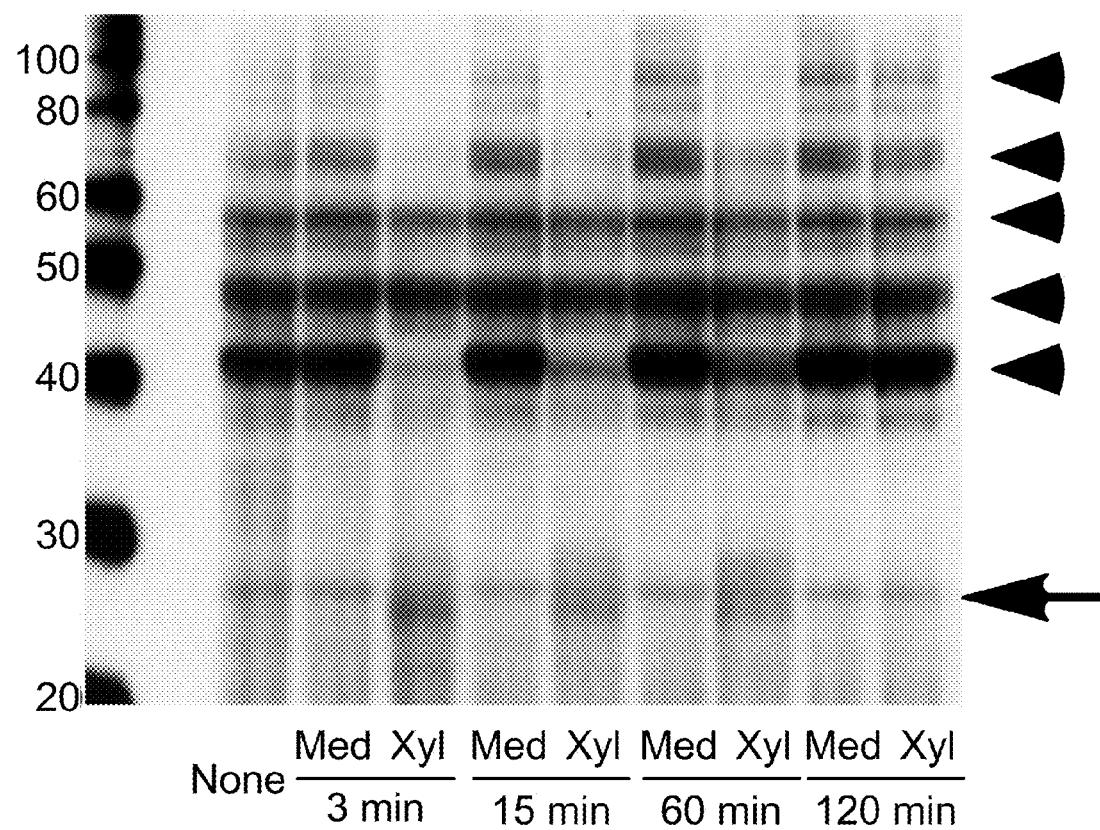


FIG. 24

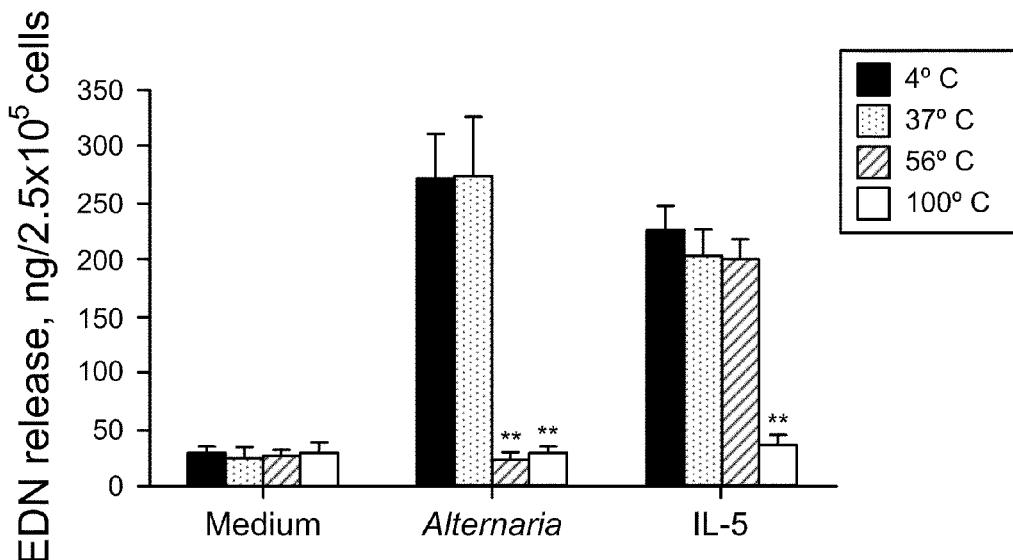


FIG. 25A

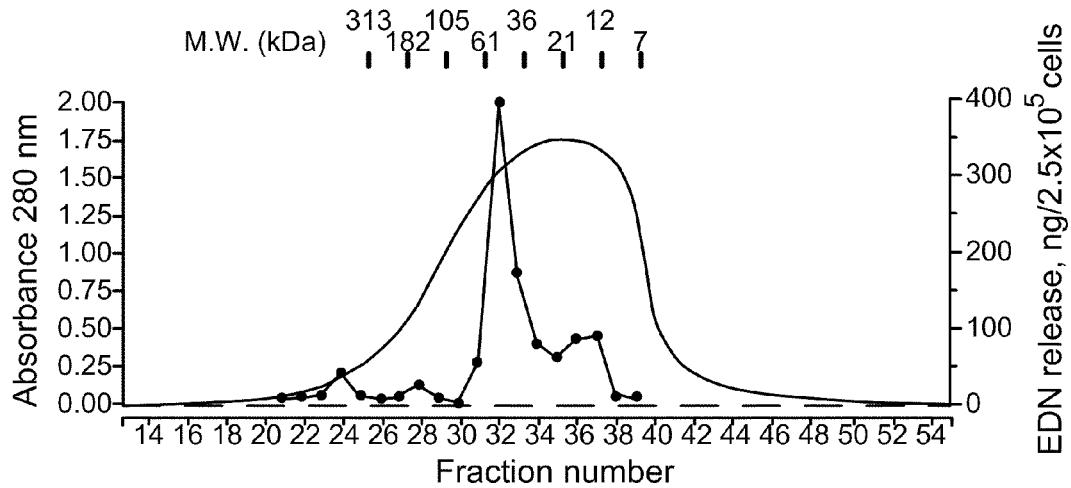


FIG. 25B

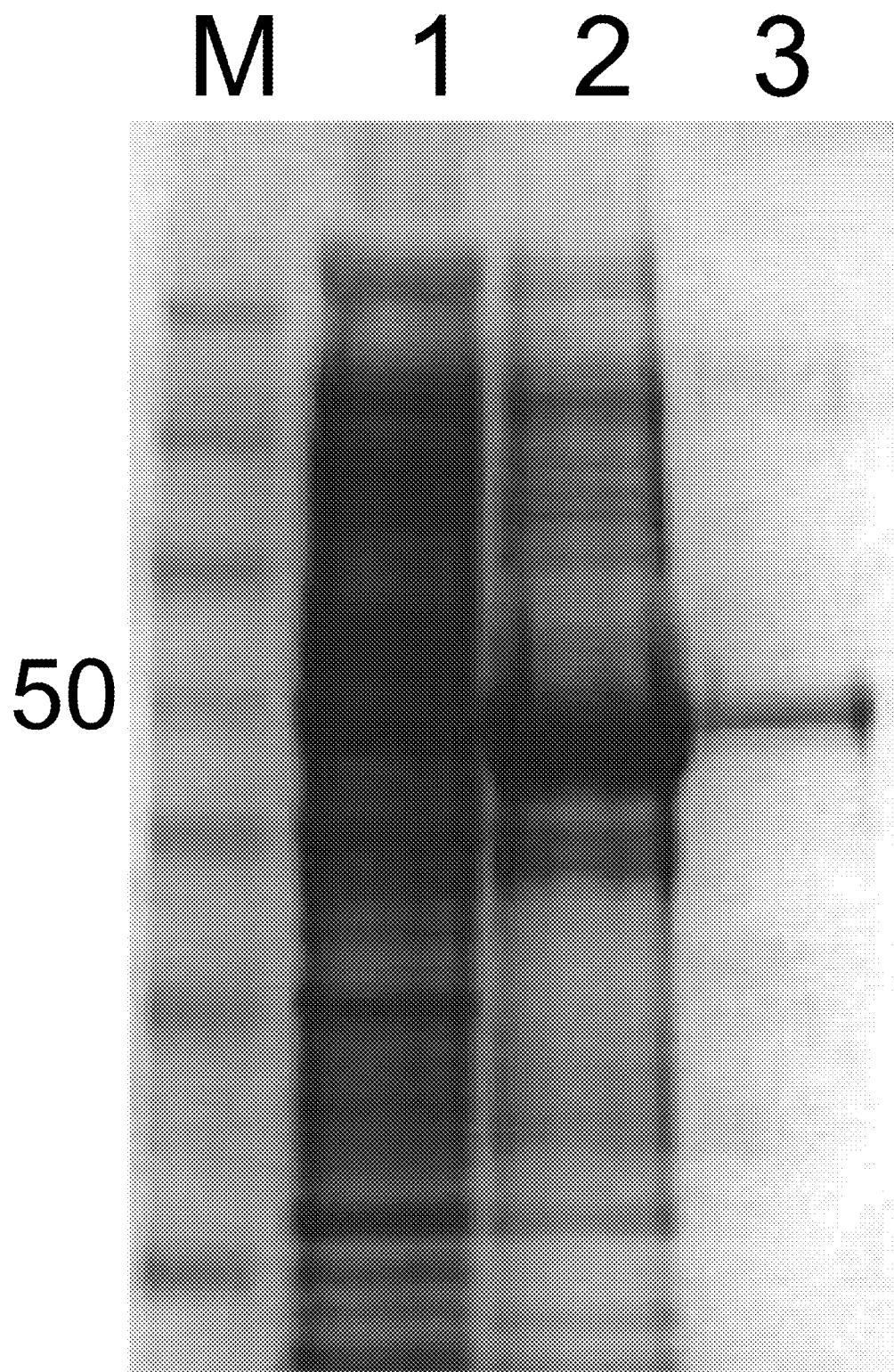


FIG. 26

FIGURE 27

ATGCATTCCGCGGATCCTCCATCTACTTCGGCATCGTGCCTCTCCTCGACT
TCAGCTGTCCCTGGAGCCGTCGCTCCCTACGGACAATGCGGTGGTAACGGCTT
CCAGGGCGAGACCGAGTGCGCTCAAGGCTGGTCTGCGTCAAGAGCAACGAC
TGGTACAGCCAGTGCATCAACGGTGGAAACGCCCGCTCCTCTGCTG
CTACTGGCGTCGCCGGCACCCGTATTCTGCGCCCGCTGTACCGTCG
ATGAACGCTAGCGAGCCCGTGCCTGCCGCCCCGTGCGGTTGCTCAGCCTGCTG
CACCGCGGTGCCAACGGCTCTGCTCTGATGTTGCCGGAACCGGTGCCAAC
GGTCCAAGTGCTCGCTCGATGCTGATTCAAGTCGACGGCAAGAAGTACA
TCGGTGTGCTACCGACCAGGGCGACTCAGCAAGGGAAAGAACAAAGGAGA
TCATCGTCGCAAACCTCGGCCAGGTTACTCCTGAGAACAGCATGAAGTGGGA
TGCCACCGAGGGTACCGAGGGCAAGTTACTCTCGACGGTGCCAACCGCCTC
GTCAGCTTGCCACGGAGAACAGCTCGTCCCGGGTACACCCACCGTCT
GGCACTCTCAGCTTCCCACCTGGGTCTCTTCCATCACCGACAAGACTAACGCTC
GAGGAAGTCATGGTGCTCACATCAAGAACGCTCATGAGCACCTACGCCGGCA
AGGTCTATGCTTGGGACGTAGTCACGAGATCTCAACGAAGACGGTTCTTC
CGCTCTCCGTCTTACAACGTTCTCGGTGAGAACTTGTGCTACCGCTTTC
GCTACTGCCAAGGCCGCCACCCAGAGGCCAAGCTCTACATCAACGACTACA
ACCTCGACAGCCCCAGTTACGCTAACGACCAAGGCCATGGCTAGCAACGTCAA
GAAGTGGGTTGCCGCCGGTGTCCATTGACGGTATTGGTCCCAGTCCACT
TGTCCGGCAGCTGCCACTCTCGACTACCCCGCTGCTCTCAAGCTTCTGC
GAGTCTGCTCCGAGTGCGCCATGACTGAGCTGACATCAAGGGTGGTGTG
CCGCTGACTACAAGACTGCTGTCAGTGTGCTTGGATGTCAGAACTGTGTT
GGTGTACCGTCTGGGTGTAGCGACACTGACTCTGGATCGCGCTGCTGC
CACTCCTCTGCTTTCGACGGCAGCTCCAGGCCAAGGAGTCTTACAACGGTC
TCTGCTCCGCTCTGCTTAAATGCACAGGGTGAGAACGAGGGCATCCGATTA
GATCTATCAGCTTAAGACAGACAATTGGTGCTGAAAAAGGTGTTGTTCT
TGTAGGAGATGGGATGAAATTCTACCGTATATATCTACTTGGTAAGATGG
TAAACTCCATCTCCAATTGATCATTATTGAAAAAAAAA (SEQ ID NO:1)

MHFRGSSIYFGIVALSSTS AVLGA VAPY GQC GGNGF QGETECAQGWSCVKSND
WYSQCINGGNAPAPPAATGVAPAPVIPS A PVP SMNA SEPVA APVAVA QPAAT
GGANGSAPDVAGTGANGAKCSLDAAFKSHGKKYIGVATDQGALSKGKNKEIIV
ANFGQVTPE NSMKW DATEGTEGKFTLDG ANALVS FATE NKKL VRGHTTVWHS
QLPTWVSSITDKT KLEE VMVA HIKKL M STYAGK VYAW DVVNE IFNEDGSFRSSV
FY NVLGENF VATA FATAKAADPEAKLYINDY NLDS PSYAKTKAMASNVKKW
AAGVPIDGI GSQSHLSGPISDYP AALKLL CESASECAMTEL DIKG GAAADYKT
AVTA CLDVENCVGVTWGVSDTD SWIGAAATPLLFDGSFQAKESYNGLCSALA
(SEQ ID NO:2)

FIGURE 28

ATGTCTCCCCGCCACAAGTTCAAGGTGCCGACATCAGTCTGCGCGTTCGGTCGCCGAGATTGAGCTCGCCGAGAATGAGATGCCTGGTCTGATGGAGACTCGCCGCAAGTATGCTGAGGACCAGCATTGAAGGGCGCCGCATTGCTGATGTCTGCACATGACCATCCAGACTGCCGTTCTCATCGAGACGCTCAAGTCCCTCGGTGCTGAGCTCACCTGGACATCCTGCAACATCTCTCCACCCAGGACCA CGCTGCCGCTGCCATTGCCGCTGCCGGCTACCTGTCTCGCCTGGAAGGGCGAGACCGAGGAGGAGTACGAGTGCTGAGCAGCAACTCACAGCTTCAA GGACGGCAAGAGCCTGAACCTGATCCTGACGACGGTGGCAGCCTCACTGCCCTTGCCACAAGAAGTACCCCTGAGATGCTCAAGGACTGCTACGGTGTCTCGGAAGAGACCAACCAGTGGTGTCCACCACCTCACCGCATGTTGAAGGGCAAGGGTCTCCTCGTCCCCGCCATCAACGTCAACGACTCCGTACCAAGTCCAAGTCCGACAACATTGTACGGTGTCCGAGTCGCTCGTACGGCATCAAGCGTGCACCGACATGATTGCTGGCAAGGTGCCGTCGCTGGTTTCGGTGTGATGTCGGCAAGGGTTGCCGCCAGGCTCTCCACAGCATGGGTGCCGTGTCATGTCACCGAGATTGACCCCATCAACGCCCTCAGGCTGCCGTTCCGGCTCCAGGTTACACCATGGAGAAGGCCGCTCCTCAGGGTCAGATCTCGTCACCACCACTGGTTGCCGTGACATCCTGACTGGCGTCCACTTCGAGGCTATGCCAACGATGCCATCGTCTGCAACATCGGTCACTCGACATCGAAATCGACGTTGCGTGGCTCAAGAAGAACGCCAAGTCCGTACCAAGCATCAAGCCCCAGGTCGACCGCTACCTGATGAAACAATGGCCGCTACATCATCCTCCTCGCTGAGGGCCGTCTCGTCAACTGGGATGCGCCACTGGCCACTCTCCTCGTCATGTCCTGCTCTTCACCAACCAAGGGTCTGCCAGATTATGCTGTACAAGGCCTCTGACGAGGAGTTGGCAACAAAGTACGTCGAGTCGGCAAGACCGGTAAGCTCGATGTCGGTGTACGTTCTGCCAACGATTCTCGACGAGCAAGTCGCTCTCCACTGGCACACGTCAACGTTGAGCTCTCCAAGCTCAGCGATGTCAGGCCGAGTACCTTGGTCTCCCTGTGAGGGCTTCAAGAGCGACATCTACCGTTACTAG (SEQ ID NO:3)

MSAPAHKFVADISLAAFGRREIELAENEMPGLMETRRKYAEDQPLKGARIAGCLHMTIQTAVLIETLKSLGAELTWTSNCNIFSTQDHAAAIAAAGVPFAWKGETEEEYEWCLEQQLTAFKDGSNLNLDDGGDLTALVHKKYPEMLKDCYGVSEETTGVHHLRYMLKGKGLLVPAINVNDSVTKSKFDNLYGCRESLDGIKRATDVMIA GKVAVVAGFGDVGKGCAQALHSMGARVIVTEIDPINALQAAVSGFQVTTMEKA APQGQIFVTTGCRDILTGVHFEAMPNDAIVCNIGHFDIEIDVAWLKKNAKSVTSIKPQVDRYLMNNGRYIILLAEGRLVNLGCATGHSSFVMSCSFTNQVLAQIMLYKA SDEEFGNKYVEFGKTGKLDVGVYVLPKILDEQVALHLAHVNVELSKLSDVQAEYLGLPVEGPFKSDIYRY (SEQ ID NO:4)

FIGURE 29

ATGAAGTCTGTAGCTGCTCCCCGCATCTTGGCCCTGGCCCACGCCACGC
CACTTCCAACAACACTGGAAAGAACGGAAAGGATCTGGAGAGCACCTGTGCC
AGGTTGCCACCCTGCAACAGCCCTGTGAGGACTACACCAGCAACGCTCTGC
AATGCAACGTCAGCCCTGCTCCTGCCGAGGGAAAGTGCCTTGAGGCCGG
TGACACGGTAACCATCGAGATGCACCCAGCACAAACACCCGTGACTGCAAGGA
GGAAGGTATTGGTGGTGCCACTGGGGCCCTGTCCCTCGCATACATGTCCAAG
GTTGAGGACGCAGCCACCGCAGATGGCTCCAGCGAGTTCTCAAGGTTACC
AGAACACCTGGCTAAGAACCCAGACGCCACTCAGGGCGACAACGACTTTG
GGGTACCAAGGACCTCAACTACAACACTGCGGAAAGCTCGACTTTGCCATTCCC
AAGAACATTGCTCCTGGTACTACCTCCTCCGTGCCGAGGCCATGCCCTCCA
CGCTGCAAGCGCAGGAGGAGCGCAACATTATATGACGTGCTTCCAACCT
ACTGTCACCGGCAGCGGAACACTGGAGCCAAGGGTGTACCTTCCCTGAGG
CGTACTCCAAGACTGGTCTCGGTCTGGTTCTCCATCCACGCCGACCTCGAC
TCATACCCTGCTCCTGGTCCCAGCTCATCCAAGCGGTACTGAGGTACCCCT
CAGCTCCTCACCTTGGCGAGCTCGCTGGTCCCCCTGCTGCCACCGCCACCGG
TGGTGCCGCCAGACCCGGCTGCTCCACCCGCTCGCTGTCTTC
ACC (SEQ ID NO:5)

MHQHNTRDCKEEGIGGAHWGPVLAYMSKVEDAATADGSSEFFKVYQNTWAKN
PDATQGDNDFWGTKDLNYNCGKLDFAIPKNIAPGDYLLRAEAIALHAASAGGG
AQHYMTCFQLTVTGSGLPDKVTFPEAYSKTGLGLFSIHADLDSYPAPGPELI
QGGTEVTPQLLTGFELAGAPAATATGAAETPAASTPASVAVSSTVAPATSSAA
AEAEPPSVAPVEVSTAVESSVAASSVAASSVVASSVAASSVAASSAASSAAASSA
AAPAESEVAPPTPEVSSVVAPYPVANSTSSMLPGTASPIVTSSIVAAPTTMLTAV
RPTQTAEASGPIKEYYQCSGQGFKGTGECAEGLECREWNSWYSQCVKPEATKLG
PSKGPMPSATASKPTATAVAPKPTVEAPKPTAETPKPSAEPTEAAAAAAEAEP
TSVEPVAVEPSKPATSSAPAAGAGEKTYTLETFIAFLEQEAGSESAAKIRRMIEAL
Q (SEQ ID NO:6)

FIGURE 30

ATGGCACCAAATAACAGGTGCCGTTGACAGCACCACAGTGAGGTATAAAAGG
ACCAAGTCGAATGGTCCCCGAGGATGTCCAGGCAGCACTGACTGGTTCA
GCACAACATCATGTCGCGCTCAAGCTTCTACAAGTTCAACACTGCTCTCC
TCCTTCTGGCACTGACAGCAGGCCAGACACCTGTCAGTCATCCGATGGCG
GTTGGAGCACCCTGCTGGCACACCTACCGCGTTCGCTCCGTTACT
CTCCCTCCCTCAGTGGACCAGGGCGTTGAGCAGATCCCCAACATCTACGATC
CGCAAGCTGTCAACCGCAGGATGTCTGCCAGGCTACAGGGCATCCGGTCT
TGAACAAGGCCATCGTGGCTGAGCGCTACCTGACGCTGGCTGGAGCTGCC
TGCAATGCTTACGGCACCGATATTGAAGAGCTGGACCTGAAGGTTGAATATC
AATCAAAGGAAGGCTGGCTGTCAAGCATTGACCCAAACATCTTGATGCTAG
CAACCGATCCAATGGATTGTGCCCGAGGATCTCATCCCGGGCCGCAAGCC
GAAGACTCGTCTGAGGGCACAGACCTCAAATTGACTGGGCAACGAACCAT
CCTCTGGTCAGTGTGGCGCTCGCTACGGGAGATGTCATCTCACCACC
CAAGGCACGAAGCTCATTATGAGAACCAATTGTTGAGTTGTCAATAACCT
GCCGAGGACTACAACCTTACGGTCTCGGAGAACGATTACGGACTTCGT
CTGAATAACAACCTCACTGCCACCATCTATGCTGCCATGTTGGTACCCAAT
CGACCGCAATCTGTACGGTAGTCACCCCTTACCTAGAAACACGCTACTTG
AAAAAGGCAGCAATGGTAGCAAGACGCCCTGAAGCAGTCTGAGCTCCAAC
AGCCCACCTTGGCTATGAAAGCAAACCAAGCTGGTTCGCCGTACGAGTCGCG
CTCTCACGGTGTACTACCGCAACACGCACGGCATGGATGTCGTTATGAAG
CCTGACCATCTCACATGGAGAACATTGGAGGTGCAATCGATCTATTCTCTA
CGAAGGACCTCTCAACCAGAAGTGACCAAGGAGTACCAAGAAGTCGGCGAT
TGGACTGCCTGCCATGCAACAGTACTGGACATTGGCTCCATCAATGCCGAT
GGGGATACCGTAATTGGACAGAGACGAGAGAGATTGTTGAGACTATGAGGG
CCTCAACATTCCCATGAAACAATTGGCTCGACATCGATTACATGGATCAA
TACCGAGACTTCACGCTTGTACCGCTTGTGGCTTCATCAGATGTCAAGGA
CTTCTTGACTGGCTCCATGGGAACAAACCAAGCAGTACCTGACCTATGTTGGATG
CCGCCATCTACATCCGAACCCACAGAACGCTAGTGACGCTTATGATAACCTA
CGCTCGCGAAATGAATCTGATGTATTCCCTGAGGAATCCTGATGGTAGTCAG
TACATTGGCGCTGTGTGGCCTGGATACACCGCTTCCAGACTGGCTGTCTTC
CAACGGTGTAGCATGGGGTTAAGGAGATGGTGAATGGTACAAGGAAGTG
CCGTACAGCGGTTCTGGTCGATATGACTGAAGTCTCCTCGTTCTGCCG
TTCCTGCGGTTCCGTAATGTTACCTGAACCTGCTCATCCACCCCTCTCCCT
CCCTGGCGAGGTGGCAACGTCAATTTCGACTATCCAGAAGGCTCAACATC
ACCAACGCAACTGAGGCCGCTCGGCTCAGCCGGCTTCGAGGCCAGGCCG
CACCGGCAGCGCCTACGGAGGGAGGCTGCTACGACCAACTAGCTACTCCGATC
AACGCCTACACCTGGTGTGCGCAACGTCAACTACCCCTACAGTCATCAAC
CATGTCCAATCCGGAGCTGATCTGCTGTCCACGCAGTCAGTCCTAATGCAAC
ACATCAGAATGGCGTTGAAGAGTACGATGTACACAACCTTATGGTCACCAG
ATCATCAATGCCACCTACCAAGGGCTTCTTCAAGTCTTCTGGAAAGCGCCC
GTTTATCATCGGACGTTCCACCTTGTGGTAGCGGAAAGTGGGCCGGTCACT
GGGGTGGTGACAACGCGTCCAAGTGGCTTATATGTTCTTTCGATCCCTCAG
GCTCTGTCGTTCTCGCTTCCGTTACCCATGTTGGGGCCGACACTGCGG

FIGURE 30 (CONTINUED)

ATTCAACGGCAACACTAATATGGAACCTTGCCTCGCTGGATGCAGCTTCCG
CCTCTTCCCCCTCTACCGCAACCACAAACGTCTTCTGCCATCCGCAGGAG
CCCTACCGCTGGGACGCCGTAGCTCTGCATCCAGGACCGCGATGCACATCC
GATACTCGCTACTACCATACTACATGTACACCCTTCAACGACGCCACACC
GGCTCGACCGTCATCGTGCGTAGCGTGGGAATTCCAATGAGCCTCAGC
TCGCAGGTGTTGACACACAGTTCATGCTGGGTCTAACATCCTAATTACTCCT
GTTCTGAGCCCCAGGTCGACACTGTTAATGGAGTATTCCCTGGTATCATCGA
CGGCAGAAAGCTGGTTCGACTGGTACTCTGGTGAGCGCGTCGAGGCCGAGGCT
GGCGTCAACACCACCATCTCTGCTCCTGGGTACATCCCCGTGTACATTG
CGGTGGCTCAGTACTACCGATCCAAGAACCTGGTACACCACGACTGAGTCC
CGCAAGAACCCATGGGTCTCATCGTGCCTTCAGCGGATGGTACTGCTTC
CGGTAACTCTGACGTCGATGACGGCGAGTCTCTCGAGCCAGAACATGTGCTT
GATGTTACGTTGCTGCTATGAATGGACAACGTAAGGCCGATGTTGAGGGAA
AGTTCAAGGACACGAACGCGCTTGCAACGTGACCATTCTGGGTGCTCCTTC
AGTTGGACAGGTCAAGTTGAATGGCGAGACAATCGATGCAAGCAAGGTGAG
CTACAACCTACTAGCAGCGTCTGAAGCTGTCAGGCTGAACGACTTGACTA
GTGGAGGAGCTGGCAGGGAAAGCTGGACTCTAAGCTGGAGTAA (SEQ ID
NO:7)

MAPNTGAVDSTTVRYKRTKSQWVPEDVQAALDWFSSTIMRSSFLQVSTLLSSF
LALTAGQTPSSSDGGWSTTLAGPTAFRSVFTLPPSVDQGVEQIPNIYDPQAVN
AQDVCPGYRASGLEQGHGRGLSATLTLAGAACNAYGTDIEELDLKVEYQSKGRL
AVSIVPKHLDASNQSQWIVPEDLIPRPQAEDSSEGTDLKFDWGNEPSFWFSVGRR
STGDVIFTTQGTKLIYENQFVEFVNLPEDYNLYGLGERIHLRLNNNFTATIYAA
DVGDPIDRNLYGSHPFYLETRYFEKGNSKPLKQSELQQPNLGYESKPAGSPY
ESRSHGVYYRNTHGMDVVMKDHTWRTLGAIDLFFYEGPSQPEVTKEYQKS
AIGLPAMQQYWTLGFHQCRWGYRNWTETREIVETMRAFNIPMETIWLDIDYMD
QYRDFTLDPVSFPPSDVKDFFDWLHGNNQHFVPIVDAAIYIPNPQNASDAYDTYA
RGNESDVFLRNPDGSQYIGAVWPGYTVFPDWLSSNGVAWWVKEMVEWYKEVP
YSGFWVDMTEVSSFCVGSCGSGNVTLNPAHPPSLPGEVGNVIFDYPEGFNITNA
TEAASASAGASSQAAPAAPTEEATTTSYFRSTPTPGVRNVNYPYVINHVQSGA
DLAVHAVSPNATHQNGVEEYDHNLYGHQINATYQQLLQVFPGKRPFIIGRSTF
AGSGKWAGHWGGDNASKWAYMFFSIPQALSFLFGIPMFGADTCGFNGNTNME
LCARWMQLSAFFPYRNHNVLSAIPQEPRWDVASASRTAMHIRYSLLPYMYT
LFNDAHTTGSTMRALAWEFPNEPQLAGVDTQFMLGPNIITPVLEPVQDVTVNG
VFPGIIDGESWFDWYSGERVEAEAGVNTTISAPLGHIPVYIRGGSVLPIQEPMYTT
ESRKNPWGLIVALSADGTASGNLYVDDGESLEPESCLDVTFAAMNGQLKADVE
GKFKDTNALANVILGAPSVGQVKLNGETIDASKVSYNSTSSVLKLSGLNDLTSG
GAWQGSWTLSWE (SEQ ID NO:8)

FIGURE 31

ATGAGGTACACTGCCACCTTCACAGGTGTACTAGCCATGCCCGGTGTCAGCG
CGTGGTCAGTATCCAGCCTTCCATTGAGGGCAACGAGGTTGTCGAGCAT
CTCCATACGGTACCAAGAGGGATGGAGAGAGGTTGGTGCCTCAGCGCCTGAGC
ATAAGCTGCATTCCGCATTGCAGTGCGCTCGGCCAACCGCGATGTATTGAA
AGGACGCTATGGAGGTTCGACTCCTAGCCACCCTCGCTACGGTCAGCACC
TAAAGCGAGACGAACCTGAAGCATCTCATCAAGCCTAGAGCCGACTCGACTGC
AAGTGTGCTTACCTGGCTCGAGCAATCCGGTATCGAAGCGCGAGACATCCAG
AACGACGGCGAGTGGATCAACTTCTGCACCCGTGAAGCGCGCCGAGCAGA
TGATGGGTACCACGTTCAAGACCTACCAAGAGTCAGCGTCCAGCGCTCAA
GAGAACCTCGCTCGTGGGTAACGATCTGTGCCCTGGACGTCCGAGTCATATTG
ATATGATCCAGCCTACCAACTCGCTTCGGTGAATCCGCCCCGAGTCAGCCA
AGTCCTTACGCAAAGACCGCTCCCTCTCGGTGTTGCTGTCATGCCACGT
GCAACACAAGGATCACGCCGATTGTCTCGCAGATCTGTACAACCTCAAGGA
TTACAACGTTAGTGACAAAGCCGATGTGACAATCGGGGTGAGCGGCTTCCTC
GAGCAGTACGCCGGTTAACGATCTGACCAAGTCTCATCCAAAGATTGCTC
CCAGCCTTGCGGTAAAACGTTCAAAGTCCAGTCTATCAATGGTAAGATGCA
GTCATTGTTACCTCGCTATCTCAGCTAACGTTGTAGACGGGCCGTTCCCTC
AAAACCTCAACGGCAACAGCGTTGAGGCTAACCTCGACATCCAGTATACAGC
TGGCTGGTGTGCGCTAACGATTTCAACCAACTTCTACACTGTTCCAGGACGAG
GACTGTTGGTCCCCGACCTTGACCAACCTGATCTCGAGGACGAGGAGCTGCC
TGAAGTACTGACGACGTCGTACGGTGAGACGGAGCAGAGCGTTCTGCCAG
TATGCCAAGAAGGTTGTGACATGATCGGCCAGCTCGGTACTCGTGGTGTCTC
GGTCATCTCGAGGATGAATCCACCAAGCCAGCGGTGATACTGGTCCAGGC
TCTGCCTGTCAGAGCAATGACGGCAAGAACGCTACCCGTCTCAACCAATCT
TCCCAGCTTCATGCCCTACGTTACTTCAGTCGGTGGCACGTTGGAGTGGAA
CCCGAACGTGCTGTTGAGTTCTCTCGGTGGCTCTGTGATCTCTGGTCTCGC
CCGGCGTACCAAGAGAAGGAGTGAACGACTACCTGGCAAACGGCTCGC
AATGGCAAGGTTGTACAACGCCAACGGACGAGGTTTCCAGATGTCGCGGC
TCAAGGAAAGGGATTCAGGTCATTGATAAGCTTGGCTGTCGTTGGAG
GAACCAGCGCCTCAGCGCCTGTCTCGCTCGGTATTGCGCTCTGAACAC
GCTCGTTGGCGGCTGGTATGCCCTCGCTGGCTTCTGAACCCTGGATCTA
CGAGCAAGGCTACAAGGGCATGAATGATATTGTCGAGGGAGGCTCGCGCG
ATGCACTGGTCGCTCTATCTATTCCGGGCTCCACGCGACTCGTGCCTTACG
CCTCCTGGAATGCGACCGAGGGCTGGATCCGTACCGGTTACGGTACACC
CGACTTGTGAGCAGATGCTCGCCTCTGACTACGCCCAATACGGTGCGCGTC
GCCTCGCGTGGTAGCCTCCGTGGAGAGGCTTAG (SEQ ID NO:9)

FIGURE 31 (CONTINUED)

MRYTATFTGVLAIAVGSAWSVSSPFHIEGNEVVEHLHTVPEGWREVGAPAPEHK
LHFRIA VRSANRDVFERTLMEVSTPSHPRYQQLKRDELKHLIKPRADSTASVLT
WLEQSGIEARDIQNDGEWINFLAPVKRAEQMMGTTFKTYQSQARPALKRTRSLG
YSVPLDVRSHIDMIQPTTRFGEIRPEFSQVLTQKTAPFSVLAVNATCNTRITPDCL
ADLYNFKDYNVSDKADVTIGVSGFLEQYARFNDLDQFIQRFAPSLAGKTFKVQSI
NGKMQSLLPRYLQLTFVDGPFPQNSTANSVEANLDIQTAGLVSPKISTTFYTVP
GRGLLVPDLDQPDLEDEELPEVLTTSYGETEQSVPAEYAKKVCDMIGQLGTRGV
SVIFEDESTTASGDTGPGSACQSNDGKNATRLQPIFPASCPTYVTSVGGTGFVPER
AVEFSSGGFSDLWSRPAYQEKA VTDYLGKLGSQWQGLYNANGRGFPDVAAQG
KGFQVIDKLGSSVGGTSASAPVFASVIALLNNARLAAGMPSLGFLNPWIYEQGY
KGMNDIVEGGSRGCTGRSIYSGLPTRLVPYASWNATEGWDPVTGYGTPDFEQM
LRLSTTPQYGARRVRRGSLRGEA (SEQ ID NO:10)

FIGURE 32

ATGGCTCCTGTGCTCTCGTTACGTGGCTCGCTGTTGGCCTTCAGGCCCTC
GCCGAGCCATTGAAAAGCTTTGATGTCCCAGAGGGATGGAAGCTCCAAG
GCCCTGCATCGGCTGCGCACACGCTAAGCTCCAGGTCGCGCTCCAGCAAGG
CGATACCGCCGGCTTGAGCAGACCGTCATGGAAATGTCCACCCCCCTCCAAT
GCAAAGTACGGGCAGCACTTGAGTCCCACGAGCAAATGAAGCGCATGCTCA
TGCCCAGTGAGGAGACCCTTCCCTCCGCTCTCCTGGCTCAAGGCTGCCGGT
ATCAAGAACTTGTGAGATTGACGCCGATTGGGTGACCTCAAGACAACCCTTG
GTGTTGCCAACGAGCTCCTCAGAACCAAGTTCTCCTGGTTGTCAGCGAGGA
GAGTACGCCCTCGCAAAGTTCTCCGCACGCTCGAGTACTCTGTGCCCGACGAC
ATTGCCGACCACATCAACCTCGTTAGCCGACCACTCGATTGCTGCTATCCG
TGCAGAACACGAGACAGAGCGCGAGATCTTCGGATTGCGCTAGCCTCTTCC
CCCAACGTCACTGTCAACTGTGATGCGTCCATCACTCCCCAGTGCTTAAGGCA
GCTCTACAAGATTGACTACACTCCCACCCCAAGAGTGGCAGTAAGGCAGCT
TTCGCTCCTATCTCGAGGAGTACGCGCGCTACAGCGACCTCGCCCTTCGA
GGAGAACGTCCTCCCCAGGCTGTGGGCCAGAACCTCTCGTTATTGAGTATA
ACGGCGGCTGAACGACCAAGCCTCTGCCGACGACAGTGGCAGGCAACTT
GGATTGCACTGATGCTCGGTCTGCCAGCCCCCTGCCGTATTGAGTATA
GCACTGGTGGACGTGGCCATGGATCGCTGACCTCGACCAGCCTGACGAGGC
TGACAGGCCAACGAGCCCTACCTCGAGTCCCTCAGTCGGTCTCAAGCTCC
CACAGAGCGATCTCCCCAGGTACCTCCACGTCTACGGCGAGAACGAACA
AACCGTACCCAAAGTCTTACGCTCTCAGCGTCTGCAACCTCTCGCTCAACTT
GTAGCCGTGGTGTCTGTCATCTTCTCATCTGGTGATTCCGGTACCGGATCC
GCCTGCCTTCCAACGACGGCAAGAACACTACCAAGTTCCAGCCTCAGTACC
CCGCTGCCTGCCATTGTCACCTCCGTCGGGTCAACTCGTACCTAACGAG
ACTGCCACTTCTCTCCTCTGGTGGTTCTCGACTACTGGAACGCCCCAG
CTACCAGGATGATGCCGTCAAGGCATACTGCAACTCGGCCAGAAC
AAGCCCTACTCAACCGCCACGGGCGGGATTCCGGACGTCTCGGCCAGG
GCTCCGGTTACAGGGTCTACGACAAGGGTTCTCAAGGGTACCGAGGTAC
TTCATGCTCCGCTCCGCTTCCGCGGTATCGTCGCTCTCCTCAATGACGCGC
GTCTGAGGGCCAAGAACGCTGCTTGGTTCTGAACCCCCCTGCTTACTCC
AACCCGGATGCGCTCAACGATATCGTTCTTGGTGGCAGCACAGGATGTGATG
GCCACGCGCGCTTCAATGGCAAGCCGAACGGTAGCCCTGTTATCCGTACGC
GAGCTGGAACGCCACTGCGGGATGGGACCCAGTTCCGGATTGGCACGCCA
AACTTCCCCAAGTTGCTCAAGGCTGCTCTCCGCTAGGTACAAGGCTTAG
(SEQ ID NO:11)

FIGURE 32 (CONTINUED)

MAPVLSFIVGSLLALQAF AEPFEKLF DVPEGWKLQGPASAATLKLQVALQQGD
TAGFEQTVMEMSTPSNAKYGQHFESHEQMKRMLMPSEETVSSVSSWLKAAGIK
NFEIDADWVTFKTTVG VANELLRTKF SWFVSEESTPRKVLRTLEY SVPDDIADHI
NLVQPTTRFAAIRANHETEREIFGIALASSPNVTVCNDASITPQCLKQLYKIDYTP
DPKSGSKAAFASYLEEYARYSDLALFEENVLPEAVGQNFSVVQFNGLNDQASA
DDSGEANLDLQYMLGLAQPLPVIEYSTGGRGPWIADLDQPDEADSANE PYLEFL
QSVLKLPQSDLPQVISTSYGENEQSVPKSYALSVCNLFAQLGSRGVSVIFSSGDSG
TGSACLSNDGKNTTKFQPQYPAACPFTSVGSTRYLNETATFFSSGGFSDYWKR
SYQDDAVKAYLHQLGQKNKPYFNRHGRGF PDVSAQGSGYRVYDKGSLKGYQG
TSCSAPAFGGIVALLNDARLRAKKPALGFLNPLLYSNPDALNDIVLGGSTGCDGH
ARFNGKPNGPSVIPYASWNATAGWDPVSGLGTNPFPKLLKAALPARYKA (SEQ
ID NO:12)

FIGURE 33

ATGTTGCCAAAACACTACTCTCATGAGCGCGCTGCTCAGCGCTGCACTGCCGA
GGTCATCTGGACGGTCGCTCAACGACATGACCTCCTTACCGAACTCTCCG
ACTGGTCCTCTCCAACCCCGTCGGCAGCTACCAATACTACATCCACGGTCCT
GGCTCCGTAACTGACTACGTAAACCTCGGCACCTCAAGAACCCCGCCG
ACACAGCTCCAAGCAAGGTGTCAAGATCACCATCGACGAGACTGCGAAATG
GAACGGCAAACCATGCTGCGACCGAACTCATCCCAGAGACCAAGGCCGC
ATCAACAAGGGCAAAGTCTACTACCACTTCTCCGTCAAGACAAACGGCTGAGA
ACCGGCCGACCGCCACCAACGAACACCAAGTCGCTTCTCGAGAGGCCACTT
CACCGAGTTGAAGTATGGCGCTTCTGGTTCTCGAACACCAACCTACAATGGC
ACGTTGGTGGCGTCTCCAAGTGGGACGTTGAGCTCGTAGCCGATGAGTGGCA
CAACGTTGCCTACGAAATCGACTTTGATGCCGGTCCCGTCGCATTCTGGCACT
CCACCGGTGCTGATGAGCTAAGCAGACAGCTGGTCGTTGAGGCTGCCGGTAACG
CCGACAAGGATGGTGTGAGGATTGGTTCTCAGCGGTGTTGGTAGTGGAGC
TGCTGGTGCAGGCCCCAGAAAAGCCTGTTGCCAGTGCTGCTGCACCTTCAAT
GTCGTTCTCTGCTGCTCCTGCTGCTACTACTTCAAGGCTGCTGTCGCCCCG
GTCTCCTCCAGCGCTCGGCTGTCGAGACTCTGTCGTATCCTCACTGCTGC
TGCTTCTCCACTGCAGTCCCTGCTGAGACCCGGCTGCTCTGCTGCTGC
TATTCCAGCGCTGCTCCCGTCGAGACTCCCGCCCTTCTACCTCTGCTGT
CACTCCGGTGTACACCTACTGCTGTTGGCCGCTTGACGCCAAGCTCCCCG
AGGAGTTACCATCAGCCAATTGTCGCTGGCTAAGGCTAAGACTGGCAA
GAACTAA (SEQ ID NO:13)

MFAKTLMSALLSAASAEVIWDGRFNDMTSTELSDWSFSNPVGSYQYYIHGP
SVTDYVNLGATFKNPADTASKQGVKITIDETAKWNGQTMLRTELIPEKAAJNK
GKVYYHFSVKTTAENAPTADEHQAFFESHFTELKYGASGSSNTNLQWHVGG
VSKWDVELVADEWHNVAYEIDFDAGSVAFWHSTGADELKQTAGPFDASTSSNG
ADWHLGVRLPGNADKGAEDWFFSGVGSGAAGAAPEKPVASAAPSNNVSS
AAPAATTSKAAVAPVSSAAAVETSVSSTAASSTAVPAETPAVSSAAISSAA
PVETPAASSTS A VTPVATPTAVAGSDAKLPEEFTISQFVAWLKAKTGKN (SEQ ID
NO:14)

FIGURE 34

ATGTCTACCTCCGAGCTGCCACCTCTTACGCCGCTCTCATCCTCGCTGATGA
CGGTGTCGACATCACTGCCGACAAGCTCCAGTCTCTCATCAAGGCCGAAAG
ATCGAGGAGGTCGAGCCCATCTGGACGACCCCTGTTGCCAAGGCTTGAGG
GCAAGGATGTCAAGGACCTGCTACTGAACGTCGGCTCAGGCGGCGCTGC
CCCTGCTGCCGGAGGCGCTGCCCCCTGCTGCTGGCGGTGCTGCTGAGGCCGCA
CCAGCTGCCGAGGAGAAGAAGGAGGAGGAGAAGGAGGAGTCAGACGAGGA
CATGGGCTTCGGTCTCTCGACTAA (SEQ ID NO:15)

MSTSELATSYAALILADDGVGITADKLQSLIKAAKIEEVEPIWTLFAKALEGKDV
KDLLLNVSGGGAAPAAGGAAPAAGGAAEAAPAAEEKKEEEKEESDEDMGFGL
FD (SEQ ID NO:16)

FIGURE 35

ATGGCTCACCTCAGTACACCCCTGCCCTCGCTGCCATATGCATAACAATGCATT
GGAGCCGCACATCTCAGCACAGATCATGGAGCTGCACCACAGCAAGCACCAC
CAGACGTATATCACCAACTTGAATGGTCTTCTCAAGACTCAAGCCGAAGCCG
TTTCTACCTCCGACATCACTCACAGGTTCGATACAGCAAGGCATCAAGTTC
AACGCTGGCGGCCACATCAACCAACTCTCTCTGGAAAACCTCGCTCCTGC
CAGCTCGGGTGAGGCTCAGAGCTCCGCTGCTCCTGAGCTACTCAAACAGATC
AAGGCAGACTGGGAGACGAGGATAAGTTCAAGGAAGCCTCAACACAGCTT
TGCTAGGCATCCAAGGAAGTGGTGGGATGGTGGTCAAGACCGATATAAGG
CAAGGAGCAGAGATTGTCTATCGTGACGACCAAGGACCGAGGATCCTGTTGTT
GGTAAAGGCGAAGTCCGATCTCGGTGTTGACATGTGGGAGCAGTCGCTACT
ATCTCCAGTACCAAGAATGGTAAGGCTGTTACGTCAAGAATATCTGGAATGT
CATTAACTGGAAGACGGCGGAGGAGCGTTATCTGGATCGCGCGCAGATGCT
TTCAGTGTGCTGAGGGCATCCATCTAA (SEQ ID NO:17)

MAAPQYTLPLPYAYNALEPHISAQIMELHHSKHHQTYITNLNGLKTQAEAVST
SDITSQVSIQQGIKFNAAGGHINHSLFWQNLAPASSGEAQSSAAPELLKQIKATWG
DEDKFKEAFNTALLGIQGSGWGWLVKTDIGKEQRLSIVTTKDQDPVVGKGEVPI
FGVDMWEHAYYLQYQNGKAAYVKNIWNVINWKTAEERYLGSRADAFSVLRAS
I (SEQ ID NO:18)

FIGURE 36

ATGGGCGTGATGAGTAAAAGGTTGCCAGCTGTATCGACGAGATTGAGGAAT
CCACTCTCAGCACCGAGGGCAAGGTCCAAGCCCAGACTGTTATTACGGAAGA
GCTTAAAAGCTGCTCAAGCACTGTGCGAATGCAACAGATTGCGTCTATAACG
GCTCTGACTTGCTCGTAACTCGCTGCATATCAATGAGTCTAATCAGGGCCC
TGACATGAGCATCATTAAAGAGCTGATCGCGGAGAACCGCGTCCGGTTGAGC
ACGCCACGCAAGAGCTGGTTATGGGGTGTGCGAAAAGTCGTGCTGGAGCAG
TAACGAGTGCAACTATCGCTATCGCGCGCGTACCTTATGGTACCAACGA
TTTGGTTGGCACCGCAGACTAACACCAACAGCATGCACCCCCCAGGTCATT
CCCTCGTCCAGCGCGCCAAGCGGTGACCAACCTCACAGGCGAAATCCACTC
CATCAAACCTGAGCATCTAGACCGCCGCTACCAGGAGCTCGAAGGCGCCTCT
GAATCTCACGGTCTCGAATCGACAACCTGGTCGAAGCACTGGTGCTCCCA
ATGCAGACGGCACCTACTATTCATCTATGCCAAACCTGACTGCCAACCTCCT
AGCGATATCCCGATGATCTACGCAAACCCGATGCCAGATTGAACGACTGC
GCAGCGAGCTGCAGACCATCGTAAGAATATTATCGCATGGACATTGCCT
CATGAAGCGTCTCAATAAGATCGACCAACGTGGTCTGTGA (SEQ ID NO:19)

MGVMSEKVASCIDEIEESTLSTEGKVQAQTVITEELKKLLKHCANATDCVYTAL
DLLRNSLHINESNQGPDMSSIKEILIAENAVERLSTPRKSWLWGVAKVVLGAVTSAT
IAIAAAAYLYGTNDFLAPQTNTNSMHPQVISLVQRAQAVTNLTGEIHSIKLEHLD
RRYQELEGASESHGLRIDNLVEALGAPNADGTYSSMPKPDCQPPSDIPMIYANP
DRQIERLRSELQTMRKNIHRMDIRLMKRLNKIDQRGL (SEQ ID NO:20)

FIGURE 37

ATGACAACCTTCCTCCCGCATCCGCATCTTACCGCGAGGGGACCAT
CGACAAAGGGTATATTACGTTAAAATGGCAAGATAAAGGCTATCGGCCAG
ATAAGCGAGGCTCCGCTGGACTCAGTAAAGACATACTCTAAACCAGGTACATA
CGATTCTTCAGGGTTGATTGACTGTACATCCATGCCGACAGGGCCGATCCT
GAAGCTCTACCCCAAGCCCTGCGCTTGGTGTGACTACCGTTGCGAGATGCA
CAACGAGCTGGAGAACGTACAAAAGCTGAAGAAGCAGACCATGGAGCCGA
TACTGCTTCATACAAGACAGCAGGCCAGGCCGCTACTATTGAGAATGGGTGG
CCTATACCCGTACGAGGCCACGACAAGACTCCAGAGACTGCAGCGCGA
TTGCGAAATGGCCAAAAGTACGGGATAGCGTGGTGGAGTTCTGGA
ATGGACTGGGAGAGAGATGCAACCAAATTACATCAAACACTATGCACGAAAG
CGGAACATATCATGGGACGCAATTAGCTATCCTCGTCAACTGCAAAGTA
CGATCATTGCAGAACGCAAAAAACGGGATACTTGACCGTGCACGCCCTACG
AACTGCGTACACGCTCGAGGTTCTGAATGCAGGTGTCGACGCCCTACG
CATACGTTTCGACCAGGCCAACCCAGGAACACTAGTAGATGCGTACAAA
AGAACAAACGCATGGGTCAACCCGACACTTGTGCGATAGGCAGCCTGACGAC
CGAGGGAAAAGAGCTGCAGCATCAATTGCACACGATCCCAGGGTAAAGG
GTTGATCAAGGAAGATCGTAGGCAACATGTGCAAGTGCATGGCTTGCT
GCAGAGGGAGGGAAAGTAGAACACGCATATCAAGGCGTAAAGGGCTGAGA
GAAGCGGGCATCGACATCCTGTGTGGAGCGACTCCGCGGGTCCGGCAGTAG
GGACGGCATTGGTCTATCGATGCATCACGAATTGTATCTCCTCGTAATAAG
GTGGGAATGACACCTATAGAGGTTACGCTCAGCCACAAGCCTGACCGCGA
AGCGCTTCCAATTAGGGATCGTGGTCTGGCGGAAGGGCTAACGCCGA
TTTGTACTGGTAGAAGGAATCCGCTGAAGACATTGATGCGACGCTAAAT
ATCCGCGGCGTTGGCGGGATGGCAACCTTGTAGCACGTTGAAAGCTT
GGAGCTGGTGTGAGCCTCTATTGAGTTGA (SEQ ID NO:21)

MTTFLLDIRIFTGEFTIDKGYIHVNQNGKIKAIQQISEAPLDSVKTYSKPGHTILPG
LIDCHIHADRADPEALPQALRFGVTVCEMHNELENVQKLKKQTMEPDATSYKT
AGQAATIENGWPIPVITAHDKTPETAAIAKWPKLTDRDSVVEFLEWTGREMQP
NYIKLMHESGTIMGRNFSYPSFELQSTIIAEAKKRGYLTVAHALSMRDTLEVNA
GVDSLHTFFDQPPTQELVDAYKKNAWVNPTLVAIGSLTTEGKELQHQFAHDP
RVKGLIKEDRVGNMCKCMGFAEAGGKVEYAYQGVKGLREAGIDILCGSDSAGP
AVGTAFLGSMHELYLLVNKGVMTPIEALRSATSLTAKRFQFRDRGRLAEGLNA
DLLLVEGNPLEIDATLNIRGVWRDGNLCSYVEKLGAGVEPLLS (SEQ ID
NO:22)

FIGURE 38

ATGGGCTCCGGATCGTCTGATAGCACCGAGTTCTTCCAGAGCTGGACTTGTG
GCAGAAGATGACTTTGTACTGGCTCGGAATTGTCGTACCATCTTCGTTG
GCCTGCTCAAACCTCTGGTATGACAAGAACAAAGGTCGCAAGTACAGCAAGGT
CGACAAGGGCAAACGGGCGTCGACGCCGAATGCTCGAGGCGCAGCCAGT
AACCCAGGTTCAAGAAGACACCAAAGATGAGATTCCCTTGATCCCGC
ATCCAAGCGGCATCGAGGTTGATGGCGTCTGGATCTCGGTACCAACACTC
CTGTTGGCAGTAGCCGTCTTCATCATGAGCGAACAGCTCCCCGCAACTTC
AACAACTCCCAGCTCGAGCTGCCAGCCAGTCGCCAGGGTTCAAGCCGCA
ACAGCTCGCGCGCTCTAGCTCGTTGACCGTGCCGTCTCGCCGAGCCTCTT
CCAAGCTACGACTCCCAGCACTCGAACACCACGTCCTCCGCAACGCTGCGGCC
GCCCTCGCTCGAGCAACTGCAACCACGTCCTCCGCAACGCTGCGGCC
CAGCGCCCTCGAGTCTCCAACTCTACCCGCAACTCTGCTGCTCC
CTCTCAAGCCAAACACAGCCAGTCTGCAAGCTCTGAGGCCACGACGAG
TGACGAGTCCGACTACATGCCATTGGCAAGAC (SEQ ID NO:23)

MGSGSSDSTEFFQSVDLWQKMTFVLACGIVVTIFVGLKLWYDKNKVRKYSKV
DKGKRASPEMLEAQPVTVQVQEDTKDEIPFGIRAIQSGIEVDGVWISRTNTPVGSS
RASIMSEQLPRNFNNSQLELPQPVAQGSSRNSSRAPSSFDRAVSAEPLPSYDSRAS
SPGRGHNHEGPRCSNCNHHVSRNAAAALSALESPNSTRNSAAPSPLQAKHSQSAS
SSSRRTSDESDYMAIGQD (SEQ ID NO:24)

FIGURE 39

ATGTGCGTGGATGTGTGGGTATGGGAATGGTCGGTGGCCGATGGTGTCGTTGCGTGCCTC
GCGTGGTGAAGCTCCAACGCGCGGCCATGGACGCCCGGAACTAGCCGTGCG
CTCGACTGGCCGGACCCTGGGTATGACGCGCTGGCCCCATGCCCATCAGATG
CCTCAAGAGGGAGCCCGAGACGGCAGCACCCACGAAACCGAATCCAAACG
CGAATGCCGCCCCACAACCAACAGAGCAGCCAGAGCAAGCGCAAGCACAATCAA
CACAGCCGTACAAAGAGGTGGCGGACGAGGTGGCAGGGGACGAGGGCAAG
GGCAAGGGCGAGGGCGAGGGCGAGGGCGAGGGGGCAAGCAGACAGTGAA
AGGCCTTCGCAACCAAATGCTGCCGCTCTGAATTGTGCCTCATCTGTACA
AGAACAGCGCATCGAGGAGGAAGACGTGGACGTGGGGGG (SEQ ID NO:25)

MCVDVWWWEWSVADGVVRVVKLQRGGHGRPELAVASTGRTLGMTRWPHAH
QMPQEEDGDGSTHETESQTRMPPHNQSSQSQRKHNQHSRHKEVADEVAGDEGK
GKGEGEGEGEGGKQTVKGLRNQMLPLSNLCLHLYKKQRIEEDVDVG (SEQ ID
NO:26)

FIGURE 40

ATGGCCGCCACCACTACAAATCATGGCACTAACACGCCCTAGCACAATGA
CATCCGCACCCACAATAACAGCCCAAGTTCCTGCCAACAGGCATGACCTAGG
CATCGTCGAGTCGGCTTCAGCGCGGCCAGCCAAAGCCGGCGTCGACGCC
GCGCCCATGGCCCTCATCGAAAATGGCCTCATCAAGCAATTAGAAGAAGATC
TAGAATTCTCCGTACCTACGACGGCCAAGTGCACAACACTACACCGAGCTCCA
GCCCTCCGACGACCCAGACTACCGGGCATGAAGCGCCCCAAGTTCGCCTCG
GCCGTACAAAGCAAGTCTTGACCAAGTCTACGAGCACGCCAAGTCGGGCA
AGCTGGTCTCACCCCTGGCGGCCGACCACTCCATGCCATTGGCACTGTTCC
GGCACCGCAAAGGCTATTCGCGAGCGGCTGGCAAGGACATGGCGTCATCT
GGGTCGATGCGATGCTGATATTAAACGCCGAGACGAGCGATTGGCAA
CATCCACGGCATGCCGTGCTTCTGACGGGCTGGCGACCGAGGAGCGG
GAAGATGTGTTGGCTGGATTAAAGAGGATCAGAGGATTAGCACGAAGAAG
CTAGTATACATTGGATTGAGGGACATTGATAGTGGAGAGAAGAAGATTCTGA
GGCAGCACGGGATCAAGGCCTTAGCATGATGATATTGACAGGCACGGTAT
TGGCAAATCATGGACATGGCGCTGGGTTGGATCGAACGACACGCCATC
CATCTCTCCTCGACGTCGACGCTCTGACCCCCATGTGGCGCCTAGCACC
TACGCCTGTCGCGGGCGCTGACGCTGCGAGGGCGACTTCATGCCGAG
TGCCTGCGAGACTGGTCAGCTATTGCTGGATCTGGTCAAGGTGAATCC
TAGCCTTGATGCCGAGGGTGCTGGCGACACGGTCCCGCTGGTGGATTG
TGAGGTGCGCGCTGGTGACACGCTTTGTAG (SEQ ID NO:27)

MAATTTNHGTNTPPSTMITSAPTIQPKFLPNRHDLGIVAVGFSGGQPKAGVDAAP
MALIENGLIKQLEEDLEFSVTYDGQVHNTELQPSDDPDYRGMKRPKFASAVTK
QVSDQVYEHAKSGKLVTLGGDHISIAIGTVSGTAKAIRERLGKDMAVIWVD
ADINTPETSDSGNIHGMPVSFLTGLATEEREDVFGWIKEQRISTKKLVYIGLRDI
DSGEKKILRQHGIKAESMHDIDRHGIGKIMDMAALGWIGSDTPIHLSFDVDALDPM
WAPSTGTPVRGGLTLREGDFIAECVAETGQLIALDLVEVNPSLDAEGAGDTVRA
GVSIVRCALGDTLL (SEQ ID NO:28)

FIGURE 41

ATGTACAGGACACTCGCTTCGCTTCCCTTCGCTCTCGGAGCCGCCGC
TCAGCAGGTTGGCAAAGAGACAACGGAGACACACCCCAAGATGACATGGCA
GAATTGCACTGGCACCGGTGAAAGAGCTGCACCAATAAGCAGGGTCCATC
GTGCTGACTCCAACGGCGATGGTCCCACGTACCAGCGGATACACCAACT
GCTTCGACGGCAACTCTTGGAACACGACCGCTGCCCTGATGGCAGCAGT
CACCAAGAAGTGCGCCATCGACGGTCCGATTACTCTGGCAGTACGGCAGT
ACCAACCCAGCAGCAATGCTGACTCTCAAGTTGTCACCAAGGGCTTACTC
TGCCAACATTGGTACGTACCTACCTCATGGAGAGTGACACCAAGTACCAA
ATGTTCAATCTCATCGGCAAGGAGTTCACCTCGATGTCATGTCAGTCCAAGCT
GCCTGCGGCTGAACGGTGCCTACTTTGTTGAAATGGCCGCCACGGTG
GCATGAACAAGGGCAACAACAAGGCCGGTCCAAGTACGGAACCGGATACT
GCGACTCCCAGTCCCTCACGACATCAAGTTATCAACGGTAGCCAACGT
AGAGGGCTGGAACCGTCCGACAATGACCCCAACGCCCGCGCTGGTAAGATT
GGTCTGCTGCCCCAAATGGATATCTGGGAGGCAACTCCATCTACTGC
CTACACTCCCCATCCCTGCAAGGGCACTGGTCTCAGGAGTGACTGACGAG
GTCAGCTGCGGTGATGGCGACAACCGTTACGGCGGTATCTGCGACAAGGACG
GTTGCGATTCAACAGCTACCGCATGGGTGTCGTGACTTCTACGGTCCAGGC
ATGACCCCTCGATACCACCAAGAAGATGACTGTCGTCACTCAGTCCCTCGGTT
CGGTTCCAGCCTCTGGAGATCAAGCGCTTACATCCAGGGAGGAACCGTC
TTCAAGAACTCCGACTCCGCCGTCGAAGGGCTCACTGGTAACCTCCATCACTG
AGGAATTCTGTGACCAGCAAAAGACCGTCTCGGTGACACATCTTCTTCAAG
ACTCTTGGTGGACTTGATGAGATGGGTGCTCGCTTGCCTCGGGTACGTCC
TGTCATGTCCCTTGGGACGACCATGCGGTCAACATGCTTGGCTCGACTCCA
CCTACCCCTACCGACGCTGACCCAGAGAAGCCTGGTATCGCCCGTGGTACCTG
CGCTACCGACTCTGGCAAGCCGAGGACGTCGAGGCCAACTCGCCCCGACGCG
ACTGTCATCTTCTCCAACATCAAGTTGGTCCCACGGCTCCACCTTCCGC
ACCCGCATAA (SEQ ID NO:29)

MYRTLALASLSLFGAARAQQVGKETTEHPKMTWQTCTGTGGKSCTNKQGSIV
LDSNWRWSHVTSGYNCDFGNNSWNTTACPDGSTCTKNCAIDGADYSGYGITT
SSNALTLKFVTKGYSANIGSRTYLMESDTKYQMFnLIGKEFTFDVDVSKLPCGL
NGALYFVEMAADGGMNKGNNKAGAKYGTGYCDSQCPHDIKFINGVANVEGW
NPSDNDPNAGAGKIGACCPEDIWEANSISTAYTPHPCKGTGLQECTDEVSCGD
GDNRYGGICDKDGCFNSYRMGVRFYGPGMTDDTTKKMTVVTQFLGSGSSL
EIKRFYIQGGTVFKNSDSAVERGVTGNSITEEFCDQQKTVFGDTSSFKTLGGLDEM
GASLARGHVLVMSLWDDHAVNMLWLDSTYPTDADPEKPGIARGTCATDSGKPE
DVEANSPDATVIFSNIKFGPIGSTFSAPA (SEQ ID NO:30)

FIGURE 42

ATGCTCTCCAACCTCCTCTCACTGCTGCGCTGCAGTAGGCGTGGCTCAGGC
CCTGCCCTCAAGCGACAAGTGTCTCGAGGACTACATCTACCGCCCGTGCAACG
ACCACTGCCCATCAGCAACTGGAAACCCCTCGCTGGCAAGGATTCTATG
CCAACCCATACTACTCGTCCGAGGTTACACCCTAGCCATGCCCTCGCTTGCT
GCGTCTCTGAAGCCCGCTGCTCTGCCGTGGCAAAGTCGGTTATTGTATG
GATGGACACAATGGCCAAGGTGCCAACATGGACACGTATCTGGCAGACATC
AAAGCCAAGAATGCCGCAGGTGCAAAGCTGATGGGTACCTTGTGCTTACG
ACCTGCCCGACCAGCAGTGCCTGCCCTCAACGGCGAGCTCAAGAT
CGACGACGGTGGTAGAGAAGTACAAGACCCAGTACATCGACAAGATTGCC
GCTATTATAAGGCCTACCCCTGACATTAAGATCAACCTGCCATTGAGGCCGA
CTCGTGGCCAACATGGTACCAACATGGCGTACAAAAGTGTGCGCGCC
GCTCCCTACTACAAAGAGCTTACCGCGTACGCTCTCAAGACGCTCAATTCCC
CAACGTCGACATGTACCTCGACGGTGGCACCGCTGGCTGGCTGGCTGGAC
GCCAACATTGGTCCAGCCGAAAAGTCTACGCCGAAGTCTACAAGGCCGTG
GCTCGCCCCCGCCGTCCGTGGTATCGTCACCAACGTCAGCAACTACAACGC
CTTCGCATCGGCACTGCCCTGCCATCACCAAGGAAACAAGAACTGCGAC
GAAGAGCGCTTCATCGACGCTTCGCTCCTCTCCCGCGCCGAAGGCTTCCC
TGCCCACTTCATCGTCGACACTGGACGTAGCGGTAAGCAGCCTACTGACCAAG
CAGGCCTGGGAGACTGGTGCAACGTTCGGGTGTGGCTTGTTATTGTC
TACTACCAACACCAACAATGCGCTTGTGATGCTTGTCTGGGTCAGCCTG
GTGGCGAGTCTGATGGTACTTCTGACCAATCTGCTGCTCGTACGACGGCTC
TGCAGCAAGGCCTCCGCTTGAAGCCTGCGCCGAGGCTGGTACTTGGTCC
AGGCATACTTGAGATGTTAAAGAACGCCAACCCGCTTGCATAA
(SEQ ID NO:31)

MLSNLLTAALAVGVAQALPQATSVSRTTSTARATTAPSATGNPFAGKDFYAN
PYYSSEVYTLAMPSLAASLKPAASAVAKVGSFVWMDTMAKVPTMDTYLADIKA
KNAAGAKLMGTFVYDLPDRDCAALASNGELKIDDGGVEKYKTQYIDKIAAIK
AYPDIKINLAIEPDSLAMVTNMGVQKCSRAAPYYKELTAYALKLNFPNDMY
LDGGHAGWLGDANIGPAAKLYAEVYKAAGSPRAVRGIVTNVSNYNAFRIGTC
PAITQGNKNCDERFIDAFAPLRAEGFPAHFIVDTGRSGKQPTDQQAWGDWCN
VSGAGFGIRPTTNTNNALVDAFWVVKPGGESDGTSDQSAARYDGFCGKASALK
PAPEAGTWFQAYFEMLLKNANPALA (SEQ ID NO:32)

FIGURE 43

ATGAAGACAACCTCTTCGTTCAAGCGGCTTCGCTGCTATCCACTCTTCGCT
CCTCTCGCTCTGCCAGGAGAAGTTACCCACGAAGGTACCGGGATTGAGT
TCTGGCGCCAGGTAGTCAGTGAUTCCCAGACTGCAGGAGGCTCGAGTGGGG
CTGGGTATTGCCAGCAGAGCCCAGTGGAGCCAACGACGAATACTCGGTTAC
ATTAAGGTCGCTGGAAGCGAACAGACAGGGATGGTCCGGTGTCAAGCCACG
CTGGTGGCATGGCTAACTCTCTTGCTCGTTGCATGGCCGGAAACTGATGCT
GTCAAGACCAAGTTGTCTGGCAGGTGGCTATATTGCTCCTGAAGACTACA
CTGGCAACCGCACTTGAGCCAGATCTTCACTCAGTCACCACACACACTTC
GAGATCGTGTACCGATGCGAGCACTGCTGGGTCTGGAATCAGGGTGGTGTG
AAGGCTCCAACCCCCACCAGCGAACGTCAATGTTATCGGCTGGGCCAGCA
TAACAAAATCTACGACGGCACTTGGGTCTTCCACAACAAGGGACAGTCCCTG
TTGGTGCTCCTACGGTGGATGCAAGGAACCGCAAGTACTCCGACTATGTCA
AACTGGCAGGAGGCCAGCCATCTGGTGCACCTACACCAACCTGTCCGGCCA
GCCGTCAAGCCACACCCACTCCACTGCACCGGTAAAGTGCACCGGATCCCCA
GCCCTTCAGGTTCTTGACTACATCGTCAATTGGTGGTGTGCTGGAGGTAT
CCCCATGGCGGACAGGCTTCCGAGTCTGGCAAGAGCGTTCTCATGCTCGAG
AAGGGCCGCCGTCCCTCGCTCGTTGGCGGAAAGATGGGCCCTGAATGGG
CTACCAACAAATTGACTCGGTTGACATCCCTGGTCTCTGCAACCAGATC
TGGGTTGACTCTGCAGGTGTTGCTGCACCGATATCGACCAAATGGCTGGCTG
TGTCCCTGGTGGAGGTACTGCCGTCAATGCTGCGCTTGGTGAAGCCGGTAG
ACATCGATTCGACTACCAATTCCCCGCTGGCTGGAAATCAGCGGACGTGAA
GGCGCGATCGACCGTGTGTTCAAGCGATCCCTGGTACTGATAACCCCTCCG
TGGACGGCAAGCGTTACAAGCAGGAAGGGCTTGATGTCCTATCCGGTGC
TGGTGGATGGCTGGAAGAGCGTCGCGAACGACCAACAGAACAGAA
GAATCGCACATACTCTCACTCTCGTTCATGTATGACAACGGTCAAAGGCAA
GGACCTCTCGGTACTTACATGGTTCTGCGCTGGAAAGGAAGAAACTCAAGC
TCTGGACGAACACCATTGGCTCGACGCATCGTCCGCACTGGCGGAACGGCTAC
CGGTGTTGAGCTTGAGAGCGGTGTCGGTGGTACTGGTTACTGCGGTACCGTC
AACCTCAACCCCTGGAGGCCGTGTTATTGTCCTCGGTGGAGCTTCGGATCGTC
AAAGGTTCTTCCGCAGCGGCATTGGACCAAAGGATCAGCTGAACATCGTG
AAGAACAGCGCTCTCGATGGCTCGACAATGATTGGAGAGTCTGACTGGATT
ACCTCCCCGTCGGCCAAAACCTGAACGACCAACCGATCTGTTATC
AGGCACCCCAACATCTCTCCTACAACATTACGAGGGCTGGATGCCCAT
CGAGGCTGACAAAGACCTGTACCTGGCAAGCGTTCTGGTATCCTGCCAGT
CTGCACCCAAACATCGGCCCCCTGCTGGGAAGTGATTACTGGAAGTGACGG
CATTGACCGATCGATCCAGTGGACTGCTCGTGTGAAGGCCGGCGCCAAC
GATACTCACCACTCACCATCAGCCAGTACCTCGGTACGGCTACTTCGCG
TGGTGCCTTCCATCAACGGTGTCTCAACGTGTATGTCAGCAAATCACCC
ACCTACAGAACGAGGCCGACACTGGTGTGGTGTGCGAGGTATCAAGAGCAT
GATGAAGGCCATCCAGAAGAACCCAGCCATCGAGTCCAAGTACCGCCTGCC
AATATGACAGTTGAGGCATACGTTGCCAGCCTCCCCAAGACCCAGCTGCC
GTCGCGCCAACCACTGGATCGGTACCGCCAAGATCGGAACCGACAGCGGTCT
CACGGGTGGAACCTCTGTGGTGGACCTGAACACTCAGGTGTATGGAACCGCAG

FIGURE 43 (CONTINUED)

AACATCCACGTAGTCGACGCTTCGCTTCCCTGGTCAAATTCAACCAACCC
TACATCCTACATCATCGTACTCGCAGAACATGCCGCTGCTAAGATTCTCGCAC
TTAGTGCAAGCAGTGGAGGTGGTAAGCCTCGTCGCTTGTGTCGTCGCA
GTCTCCGCTAAACCCACTACCTCGAAGGCACCAACTGAGTCGTCAACCGTAT
CCGTGGAGCGTCCATCGACACCAGCCAAGTCTCGGCTAAGTCGACTACTAT
CAAGACATCTGCAGCACCAGCACCTACTCCTACCAGGGTGTGAAGGCCTGG
GAACGATGCGGTGGTAAAGGCTACACTGGCCAACAGCCTGTGTCAGTGGC
ACAAGTGCAGTGAATGAGTACTACTCTCAGTCATCCCTAACTAA
(SEQ ID NO:33)

MKTTSFVQAASLLSTLFAPLALAQEKFTHEGTGIEFWRQVVSDSQTAGGFEWGW
VLPAEPTGANDEYIGYIKGSLEANRQGWGVSHAGGMANSLLLVAWPETDAVK
TKFVWAGGYIAPEDYTGNATLSQIFHSVTDTHEIVYRCEHCWVNQGGAEGS
QLPTSENVIGWAQHNKIYDGTWVFHNKGQSLFGAPTVDARNAKYSDYVLAG
GQPSGAPTPTLSGQPSATPTPTAPVKCTGSPAPSGSFDYIVIGGGAGGIPMADRLS
ESGKSVLMLEKGPPSLARFGGKMGPEWATTNNLRFDIPGLCNQIWVDSAGVAC
TDIDQMAGCVLGGGTAVNAALWWKPVDIFDYQFPAGWKSADVKGайдРВFK
RIPGTDTPSVDGKRYKQEGFDVLSGALGADGWKSVVANDQQNQKNRTYSHSPF
MYDNGQRQGPLGTYMVSALERKNFKLWTNTMARRIVRTGGTATGVELESGVG
GTGCGTVNLNPGRVIVSGAEGSSKVLFRSGIGPKDQLNIVKNSALDGSTMIG
ESDWINLPVGQNLDHVNTDLVIRHPNISSYNFYEAWDAPIEADKDLYLGKRSGI
LAQSAPNIGPLAWEVITGSDGIDRSIQWTARVEPGANDTHHLTISQYLGHGSTS
RGALSINGALNVYVSKSPYLQNEADTGVVAGIKSMMKAIQKNPAIEFQVPPAN
MTVEAYVASLPKTPAARRANHWIGTAKIGTDSGLTGGTSVVDLNTQVYGTQNIH
VVDASLFPQQIFTNPTSYIIVLAEHAAAKILALSASSGGGKPSSSALSSAVSAKPTT
SKAPTESSTVSVERPSTPAKSSAKSTTIKTSAAPAPTPTRVSKAWERCGGKGYTGP
TACVSGHKCAVSNEYYSQCIPN (SEQ ID NO:34)

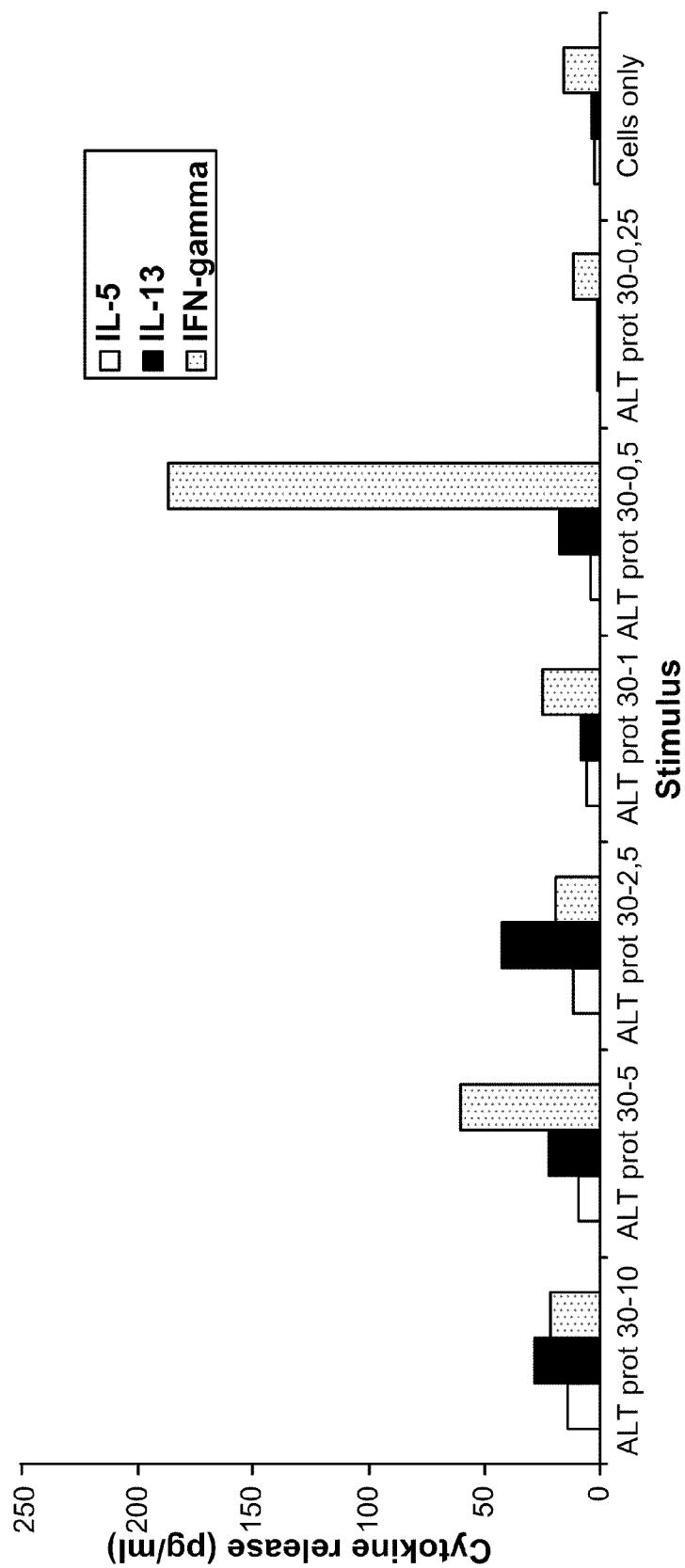


FIG. 44

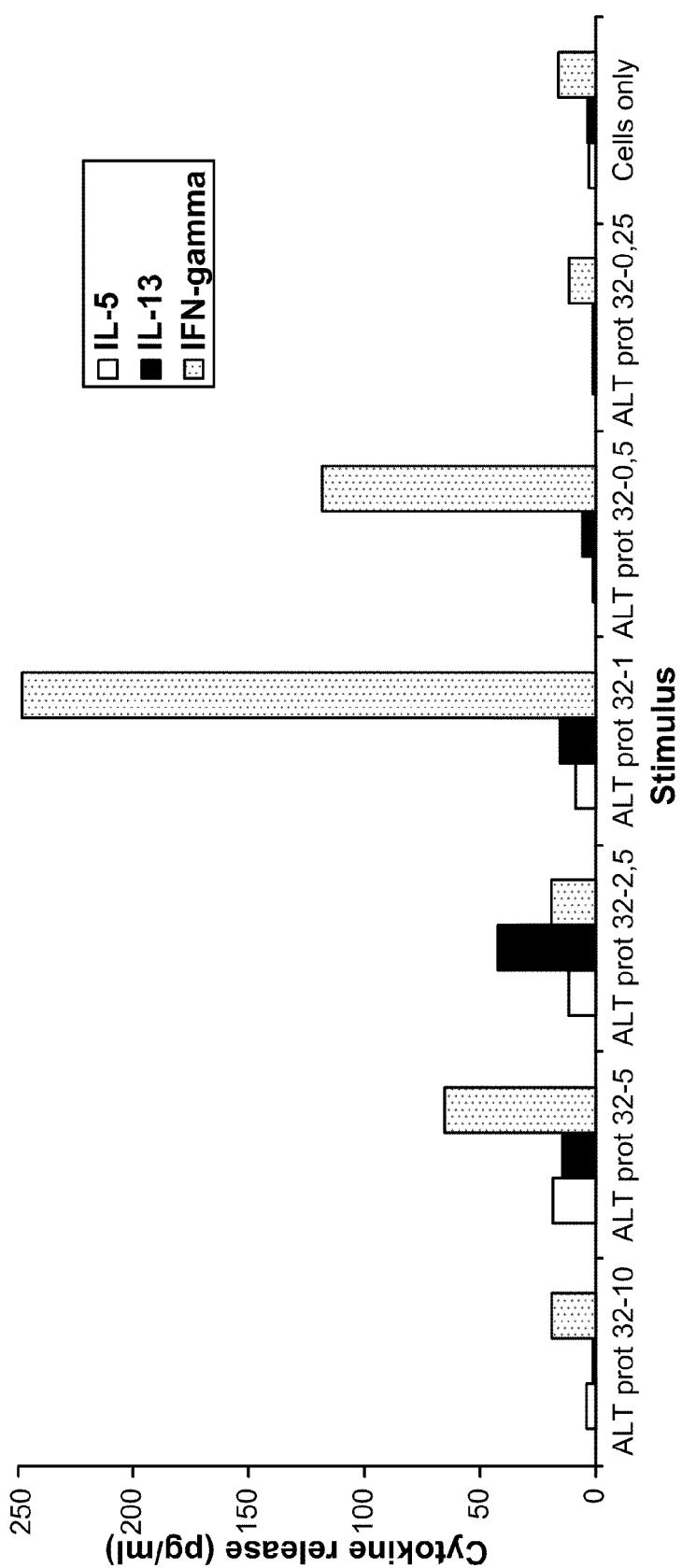


FIG. 45

FUNGUS-INDUCED INFLAMMATION AND EOSINOPHIL DEGRANULATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 12/835,592, filed Jul. 13, 2010, now U.S. Pat. No. 7,887,820 which is a divisional of U.S. application Ser. No. 12/629,638, filed Dec. 2, 2009 (now U.S. Pat. No. 7,815,919), which is a divisional of U.S. application Ser. No. 11/580,454, filed Oct. 13, 2006 (now U.S. Pat. No. 7,662,400), which claims the benefit of U.S. Provisional Application Ser. No. 60/726,553, filed Oct. 14, 2005. The disclosure of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with government support under AI049235 awarded by the National Institute of Allergy and Infectious Diseases. The government has certain rights in the invention.

BACKGROUND

1. Technical Field

This document relates to methods and materials involved in fungus-induced inflammation and eosinophil degranulation. For example, this document relates to isolated nucleic acids encoding fungal polypeptides, fungal polypeptides, methods for assessing fungus-induced inflammation, methods for assessing eosinophil degranulation, and methods for identifying inhibitors of fungus-induced inflammation and/or eosinophil degranulation.

2. Background Information

The National Center for Health Statistics describes the increasingly expensive health care burden that chronic rhinosinusitis (CRS) inflicts in the United States. With an estimated 18 to 22 million cases and at least 30 million courses of antibiotics per year, CRS is one of the predominant chronic diseases in the U.S. In 1996, there were 26.7 million visits to physicians, hospital offices, and emergency departments for sinusitis—at a total cost of \$5.8 billion. Sinusitis significantly impacts quality of life, even when compared to typical chronic debilitating diseases, such as diabetes and congestive heart failure. CRS presents a challenge to various medical specialties, including infectious diseases, ear, nose, and throat (ENT), allergy, asthma, and clinical immunology. The FDA has not approved any medication for effective use in CRS. Many antibiotic treatments are prescribed without objective evidence of infection. Roughly 40,000 patients per year undergo sinus surgery, but controlled evidence about the surgical outcomes is lacking. Even with aggressive medical and surgical therapies, many patients have persistent or recurrent disease, leading to frequent courses of antibiotics and multiple surgical interventions.

SUMMARY

This document relates to methods and materials involved in fungus-induced inflammation and eosinophil degranulation. For example, this document relates to isolated nucleic acids encoding fungal polypeptides, fungal polypeptides, methods for assessing fungus-induced inflammation, meth-

ods for assessing eosinophil degranulation, and methods for identifying inhibitors of fungus-induced inflammation and/or eosinophil degranulation.

In general, one aspect of this document features a substantially pure polypeptide comprising, or consisting essentially of, an amino acid sequence at least 95 percent identical to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34. The polypeptide can comprise the amino acid sequence set forth in SEQ ID NO:10. The polypeptide can comprise an amino acid sequence having 99% identity to the sequence set forth in SEQ ID NO:10. The polypeptide can comprise the amino acid sequence set forth in SEQ ID NO:12 or 22. The polypeptide can comprise an amino acid sequence having 99% identity to the sequence set forth in SEQ ID NO:12 or 22.

In another aspect, this document features an isolated nucleic acid comprising, or consisting essentially of, a nucleic acid sequence that encodes a polypeptide comprising an amino acid sequence at least 95 percent identical to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34. The polypeptide can comprise the amino acid sequence set forth in SEQ ID NO:10. The polypeptide can comprise an amino acid sequence having 99% identity to the sequence set forth in SEQ ID NO:10. The polypeptide can comprise the amino acid sequence set forth in SEQ ID NO:12 or 22. The polypeptide can comprise an amino acid sequence having fewer than 5 mismatches as compared to the sequence set forth in SEQ ID NO:10, 12, or 22. The nucleic acid can hybridize under highly stringent hybridization conditions to the nucleic acid sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33. The nucleic acid can hybridize under highly stringent hybridization conditions to the nucleic acid sequence set forth in SEQ ID NO:9, 11, or 21.

In another aspect, this document features a purified antibody having the ability to bind to a polypeptide comprising, or consisting essentially of, an amino acid sequence at least 95 percent identical to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34. The antibody can have a dissociation constant that is less than 10^{-7} for the polypeptide. The polypeptide can be a polypeptide having the sequence set forth in SEQ ID NO:10, 12, or 22.

In another aspect, this document features a method of identifying an inhibitor of fungus-induced eosinophil degranulation. The method comprises, or consists essentially of, determining whether or not a test agent reduces the amount of eosinophil degranulation induced by a preparation comprising a polypeptide having an amino acid sequence at least 95 percent identical to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, wherein the reduction indicates that the test agent is the inhibitor. The polypeptide can be a recombinantly produced polypeptide. The amount of eosinophil degranulation can be determined by measuring major basic protein or eosinophil-derived neurotoxin.

In another aspect, this document features a method of identifying an inhibitor of fungus-induced inflammation. The method comprises, or consists essentially of, determining whether or not a test agent reduces the amount of inflammation induced in a mammal by a preparation comprising a polypeptide having an amino acid sequence at least 95 percent identical to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, wherein the reduction indicates that the test agent is the inhibitor. The polypeptide can be a recombinantly produced polypeptide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

FIG. 1. Production of IL-5 from PBMC from normal individuals (n=15) and patients with CRS (n=18) cultured with extracts of common environmental fungi.

FIG. 2. Correlation between *Alternaria*-specific IgE (A) and IgG (B) in sera and *Alternaria*-induced PBMC production of IL-5 in patients with CRS.

FIG. 3. Serum levels of IgG4 antibodies to *Alternaria* (left) and *Aspergillus* (right) in normal individuals and patients with allergic rhinitis (AR) and CRS. Each dot represents one subject Assay sensitivity, 100 µg/L. Statistical analysis by Mann-Whitney U test.

FIG. 4. H&E (A), GMS (B), anti-*Alternaria* (C), and anti-MSP (D) staining of sinus tissue specimen from a patients with CRS. Arrowheads point to GMS-positive fungi, which are barely detectable by this staining. Also note presence of fungal organisms as detected by anti-*Alternaria* Ab (panel C) and diffuse deposition of MBP (panel D) in sinus mucus, but not in sinus tissue.

FIG. 5. Effects of fungi on eosinophil degranulation. Eosinophils were incubated with culture extracts of various fungi for 3 hours. EDN concentrations in the supernatants were measured by RIA as an indicator of degranulation. *, p<0.05 compared to medium alone, n=5.

FIG. 6. Characterization of activity in *Alternaria* extract. Panel A, *Alternaria* extracts were treated at various temperatures before incubation with eosinophils. Panel B, size exclusion chromatography with Superdex 200-10/30 column.

FIG. 7. Mechanism of PAR-2 activation.

FIG. 8. Desensitization of eosinophil calcium response (Panel A) and EDN release (Panel B) by PAR-2 peptides. Cells were preincubated with PAR-2 agonist (SLIGKV; SEQ ID NO:38), PAR-2 antagonist (LSIGKV; SEQ ID NO:35) or control peptide (GLIVKS; SEQ ID NO:36) (all at 100 µM) before stimulation with *Alternaria* extract (Panel A) or with *Alternaria* extract, PAF or PMA (Panel B).

FIG. 9. Effects of protease inhibitors on PAR-2 cleavage activity (Panel A) and EDN release activity (Panel B) of *Alternaria* extract. *Alternaria* extract, trypsin, or PMA was pretreated with pepstatin A agarose, control agarose, or APMSF, and added to the PAR-2 peptide substrate (Panel A) or eosinophils (Panel B). In Panel B, *, p<0.05 compared to no inhibitors, n=4.

FIG. 10. Effects of fungi on IL-6 production by BEAS-2B cells. BEAS-2B cells were incubated with culture extracts of various fungi for 24 hours. IL-6 concentrations in the supernatants were measured by ELISA. *, p<0.05 compared to medium alone, n=3.

FIG. 11. Effects of an aspartate protease inhibitor, ritonavir, on IL-8 production by BEAS-2B cells. *Alternaria* extract or TNF-α was pretreated with ritonavir and added to BEAS-2B cells. IL-8 concentrations in the supernatants were measured after 24 hours. Data are normalized to the values without ritonavir as 100%. *, p<0.05 compared to no inhibitor, n=4.

FIG. 12. Panel A. DEAE fractionation of *Alternaria* extract. *Alternaria* extract was separated by DEAE anion-exchange chromatography (Buffer A, 20 mM Tris pH 7.5; Buffer B, 20 mM Tris 1M NaCl pH 7.5) and individual fractions were analyzed for their PAR-2 cleavage activity, aspartate protease activity, and eosinophil degranulation activity. Panel B. A silver-stained SDS-PAGE analysis. Lane 1; crude *Alternaria* extract, Lane 2; DEAE fraction #18 further purified by hydroxyapatite chromatography.

FIG. 13. Morphology of eosinophils incubated with germinating *A. alternata* (Panel A) and release of EDN by these eosinophils (Panel B). Spores of *A. alternata* were cultured in RPMI medium with 10% FCS for 12 hours. Freshly isolated eosinophils were added to the wells at indicated eosinophil:spore ratios and incubated for an additional 4 hours. Concentrations of EDN released into the supernatants were measured by ELISA. Data are presented as mean±range from a duplicate experiment. Left panel and right panel in Panel A shows bright field image and anti-MBP immunofluorescence staining (to visualize eosinophils), respectively.

FIG. 14. Morphology of spores from GFP-transformed *A. alternata*.

FIG. 15. Growth of *A. alternata* and production of PAR-2 activating enzyme(s). Spores of GFP-transformed *A. alternata* (1,000 spore/well of 96-well tissue culture plates) were cultured in HBSS medium supplemented with different concentrations of bovine mucin from submaxillary glands. Fungal growth was quantitated after 48 hours by measuring the intensity of GFP fluorescence in each well (Panel A). Production of PAR-2 activating proteases by fungi into the supernatants was measured at 24 hours or 48 hours by using a fluorescence quenched PAR-2 peptide substrate (Abz-SKGRSLIGK(Dnp)D) (Panel B) (SEQ ID NO:37). Data are presented as mean±SEM from a triplicate experiment.

FIG. 16. Effects of intranasal exposure to fungal antigens or OVA on airway inflammation. Naive mice were exposed intranasally to antigens (250 µg/exposure) without prior sensitization. Alt (cult), *Alternaria* culture supernatant; Alt (cell), *Alternaria* cellular extract; Can, *Candida* extract; Asp, *Aspergillus* extract.

FIG. 17. Effects of immune cell deficiency on *Alternaria*-induced airway eosinophilia and early cytokine response. Naive Rag-1 knockout (Rag-1) or wild type (WT) mice were exposed to *Alternaria* (Alt) intranasally on days 0, 3, and 6. Panel A shows kinetics of airway eosinophilia. Panel B shows early cytokine response 12 hours after the first exposure (i.e. day 0.5), n=4-9.

FIG. 18. Early airway IL-5 production in response to *Alternaria* exposure. Panel A: BALB/c mice were pretreated by intranasal administration of LPS (1 µg) or PBS on day -3, and then exposed to *Alternaria* (Alt) on day 0. BAL fluids were collected 12 hours later. Panel B: C3H/HeOuJ or C3H/HeJ mice were exposed to *Alternaria* or PBS on day 0 without prior treatment. BAL fluids were collected 12 hours later. n=5-6.

FIG. 19. *Alternaria* extract was pretreated with pepstatin A-agarose (Pep A) or control agarose (Cont). Panel A: Mice were intranasally challenged with treated *Alternaria* extract on day 0, and BAL fluids were analyzed for IL-5 after 12 hours. Panel B: Mice were intranasally challenged with

treated *Alternaria* extract or PBS on days 0, 3, and 6, and BAL fluids were analyzed for eosinophil numbers on day 8. n=4-7.

FIG. 20. Effects of *Alternaria* DEAE fractions on airway inflammation. Naive mice were exposed intranasally to PBS or DEAE fractions of *Alternaria* extract without prior sensitization. The fractions used are those described in FIG. 12. n=3.

FIG. 21. Effects of *Trichoderma* xylanase on eosinophil degranulation (Panel A). Effects of *Trichoderma* xylanase on IL-5 production in mouse airways (Panel B). Panel A: Eosinophils were incubated with various concentrations of *Trichoderma* xylanase for 3 hours. EDN concentrations in the supernatants were measured by RIA as an indicator of degranulation. Panel B: Naive BALB/c mice were exposed intranasally to various doses of *Trichoderma* xylanase. After 12 hours, BAL fluids were collected and the concentrations of IL-5 were measured by ELISA. Mean±range, n=2.

FIG. 22. PBMC proliferation monitored using CFSE labeling. PBMCs from a CRS patient were isolated, labeled with CFSE, and cultured in the presence of 25 µg/ml *Alternaria* extract (Alt) or medium alone (Med). On days 4 and 7, cells were collected, stained with CD4 PE, and analyzed by FACS. Numbers represent the percentage of CFSE^{low} CD4⁺ cells among total CD4⁺ cells.

FIG. 23. Comparison of normal and CRS proliferation using CFSE labeling. PBMCs from a normal individual and a CRS patient were CFSE labeled and cultured with 25 µg/ml *Alternaria* extract (Alt), 2 µg/ml tetanus toxoid (TT) or medium alone (Med). On day 7, cells were collected, stained with CD4 PE, and analyzed by FACS.

FIG. 24. Temporary deglycosylation and downregulation of PAR-2 by xylanase. Isolated eosinophils were incubated with medium alone (Med) or *Aspergillus* xylanase (Xyl) for the indicated time. Cells were lysed and analyzed for PAR-2 molecules by anti-PAR-2 antibody (which recognizes the N-terminus of the molecule) and Western blot. The 41 kDa and 70 kDa PAR-2 molecules were deglycosylated by xylanase temporarily. Arrow; PAR-2 core protein, Arrow heads; glycosylated PAR-2 molecules.

FIG. 25. Partial characterization of *Alternaria* extract. A, Before incubation with eosinophils, aliquots of 100 µg/mL *Alternaria* and 10 ng/mL IL-5 were heated at 37, 56, or 100° C. for 30 min or were treated at 4° C. for 30 min. Eosinophils were incubated in duplicate with these treated stimuli for 3 hours at 37° C. Results show the mean±SEM from five different eosinophil preparations. B, Size exclusion chromatography used a Superdex 200-10/30 column and produced a broad absorbance peak (smooth line) of the *Alternaria* culture extract. The dots connected by lines show the levels of EDN release when portions of fractions 21-39 were incubated with eosinophils. The molecular weight calibration of the column is shown above the elution profile.

FIG. 26. *A. alternate* xylanase was PCR amplified using genomic DNA as template. PCR product was cloned in pQE-30 UA *E. coli* expression vector. The vector was transformed into the *E. coli* M15 host strain using electroporation and screened for the 6x-His tag. Strong positive colonies were selected and grown in one-liter culture. After induction with IPTG, proteins were purified by a Ni-NTA column. M; marker, 1; protein from uninduced culture, 2; protein from culture induced with IPTG, 3; following purification with Ni-NTA column.

FIG. 27. Nucleic acid sequence (SEQ ID NO:1) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:2.

FIG. 28. Nucleic acid sequence (SEQ ID NO:3) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:4.

FIG. 29. Nucleic acid sequence (SEQ ID NO:5) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:6.

FIG. 30. Nucleic acid sequence (SEQ ID NO:7) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:8.

FIG. 31. Nucleic acid sequence (SEQ ID NO:9) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:10.

FIG. 32. Nucleic acid sequence (SEQ ID NO:11) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:12.

FIG. 33. Nucleic acid sequence (SEQ ID NO:13) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:14.

FIG. 34. Nucleic acid sequence (SEQ ID NO:15) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:16.

FIG. 35. Nucleic acid sequence (SEQ ID NO:17) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:18.

FIG. 36. Nucleic acid sequence (SEQ ID NO:19) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:20.

FIG. 37. Nucleic acid sequence (SEQ ID NO:21) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:22.

FIG. 38. Nucleic acid sequence (SEQ ID NO:23) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:24.

FIG. 39. Nucleic acid sequence (SEQ ID NO:25) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:26.

FIG. 40. Nucleic acid sequence (SEQ ID NO:27) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:28.

FIG. 41. Nucleic acid sequence (SEQ ID NO:29) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:30.

FIG. 42. Nucleic acid sequence (SEQ ID NO:31) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:32.

FIG. 43. Nucleic acid sequence (SEQ ID NO:33) encoding a fungal polypeptide having the amino acid sequence set forth in SEQ ID NO:34.

FIG. 44. PBMC after challenge with isolated *Alternaria* protein fractions 30.

FIG. 45. PBMC after challenge with isolated *Alternaria* protein fractions 32.

DETAILED DESCRIPTION

This document relates to methods and materials involved in fungus-induced inflammation and eosinophil degranulation. For example, this document provides isolated nucleic acids encoding fungal polypeptides, substantially pure fungal polypeptides, methods for assessing fungus-induced inflammation, methods for assessing eosinophil degranulation, and methods for identifying inhibitors of fungus-induced inflammation and/or eosinophil degranulation. This document also provides methods and materials for making and using an antibody that can bind a fungal polypeptide. In addition, this

document provides methods and materials for treating a mammal having a fungus-induced inflammatory condition (e.g., CRS).

Fungal Polypeptides and Nucleic Acids Encoding Fungal Polypeptides

This document provides a substantially pure fungal polypeptide. Such fungal polypeptides can have the ability to stimulate eosinophil degranulation and/or inflammation. For example a fungal polypeptide provided herein can have the ability to stimulate eosinophil degranulation in vitro, can have the ability to stimulate inflammation in vivo, or both. The term "substantially pure" with respect to a polypeptide refers to a polypeptide that has been separated from cellular components with which it is naturally accompanied. Typically, a polypeptide provided herein is substantially pure when it is at least 60 percent (e.g., 65, 70, 75, 80, 90, 95, or 99 percent), by weight, free from proteins and naturally-occurring organic molecules with which it is naturally associated. In general, a substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. In some cases, a substantially pure polypeptide can be a polypeptide preparation that contains one of the polypeptides set forth in FIGS. 27-39 or a polypeptide at least about 80 percent identical to such a polypeptide, while being free of at least one of the other polypeptides set forth in FIGS. 27-39.

The polypeptides provided herein can be at least five amino acids in length (e.g., at least 6, 7, 10, 15, 30, 50, 70, or 100 amino acids in length). A substantially pure polypeptide provided herein can be a polypeptide having a sequence that is at least 80 percent identical to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34. For example, a polypeptide provided herein can have at least 80, 85, 90, 95, 98, or 99 percent identity to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26. In some cases, a polypeptide provided herein can have the exact amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34.

The percent identity between a particular amino acid sequence and the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 is determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (B12seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the State University of New York-Old Westbury Library (call number: QH 447.M6714). Instructions explaining how to use the B12seq program can be found in the readme file accompanying BLASTZ. B12seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of B12seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\B12seq c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences.

Once aligned, the number of matches is determined by counting the number of positions where an identical amino

acid residue is presented in both sequences. The percent identity is determined by dividing the number of matches by the length of the full-length amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 followed by multiplying the resulting value by 100. For example, an amino acid sequence that has 144 matches when aligned with the sequence set forth in SEQ ID NO:26 is 96.0 percent identical to the sequence set forth in SEQ ID NO:26 (i.e., $144 \div 150 * 100 = 96.0$).

It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 are rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 are rounded up to 78.2. It also is noted that the length value will always be an integer.

In some cases, a substantially pure polypeptide provided herein can have fewer than 10 (e.g., fewer than 9, 8, 7, 6, 5, 4, 3, or 2) mismatches as compared to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34. For example, a polypeptide provided herein can have 4, 3, 2, or 1 mismatches as compared to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34.

A substantially pure polypeptide provided herein can be obtained, for example, by extraction from a natural source (e.g., *Alternaria* cells), chemical synthesis, or by recombinant production in a host cell. To recombinantly produce a polypeptide provided herein, a nucleic acid sequence encoding the polypeptide can be ligated into an expression vector and used to transform a bacterial or eukaryotic host cell (e.g., insect, yeast, *Alternaria*, *Pichia*, or mammalian cells). In general, nucleic acid constructs can include a regulatory sequence operably linked to a nucleic acid sequence encoding a polypeptide provided herein. Regulatory sequences do not typically encode a gene product, but instead affect the expression of the nucleic acid sequence. In bacterial systems, a strain of *Escherichia coli* such as BL-21 can be used. Suitable *E. coli* vectors include the pGEX series of vectors (Amersham Biosciences Corp., Piscataway, N.J.) that produce fusion proteins with glutathione S-transferase (GST). Transformed *E. coli* typically are grown exponentially, and then stimulated with isopropylthio-galactopyranoside (IPTG) prior to harvesting. In general, such fusion proteins can be soluble and can be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors can be designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In some cases, fungi can be grown in large quantities in vitro, and a polypeptide provided herein that is endogenously produced can be separated and purified using chromatographic methods (e.g., HPLC and/or FPLC with a variety of separation matrices). In order to produce recombinant, highly purified forms of a polypeptide provided herein, one method would be to engineer an affinity tag (e.g. 6x Histidine tag) either on the N- or C-terminus of the polypeptide (either via manipulation of the cDNA nucleic acid sequence with PCR mutagenesis, or use of expression vectors containing an affinity tag sequence) to aid in purification. Existing *Pichia pastoris* expression vectors and purification systems like those from Invitrogen (Carlsbad, Calif.) can be used for production of recombinant fungal polypeptides. Moreover, yeast and fungi are closely related organisms and thus recombinantly produced fungal polypeptides in *P. pastoris* can have an increased chance of being properly folded and retain post translation (e.g., glycosylation) modifications involved in activity. *P. pastoris* can be used as described elsewhere (Rei-

chard et al., *Appl. Environ. Microbiol.*, 72(3):1739-48 (2006)). Another method can involve using *Alternaria* itself as a production system. This can be accomplished by engineering an affinity tag on the desired polypeptide and then employing the LME fungal transformation approaches as described elsewhere (Cho et al., *Molecular Plant-Microbe Interact.*, 19:7-15 (2006)).

In eukaryotic host cells, a number of viral-based expression systems can be utilized to express polypeptides provided herein. A nucleic acid encoding a polypeptide provided herein can be cloned into, for example, a baculoviral vector such as pBlueBac (Invitrogen, Carlsbad, Calif.) and then used to co-transfect insect cells such as *Spodoptera frugiperda* (Sf9) cells with wild type DNA from *Autographa californica* multiply enveloped nuclear polyhedrosis virus (AcMNPV). Recombinant viruses producing polypeptides provided herein can be identified by standard methodology. In some cases, a nucleic acid encoding a polypeptide provided herein can be introduced into a SV40, retroviral, or vaccinia based viral vector and used to infect suitable host cells.

Mammalian cell lines that stably express a polypeptide provided herein can be produced using expression vectors with the appropriate control elements and a selectable marker. For example, the eukaryotic expression vectors pCR3.1 (Invitrogen) and p91023(B) (see Wong et al., *Science*, 228:810-815 (1985)) can be used to express a polypeptide provided herein in, for example, Chinese hamster ovary (CHO) cells, COS-1 cells, human embryonic kidney 293 cells, NIH3T3 cells, BHK21 cells, MDCK cells, and human vascular endothelial cells (HUVEC). Following introduction of the expression vector by electroporation, lipofection, calcium phosphate or calcium chloride co-precipitation, DEAE dextran, or other suitable transfection method, stable cell lines can be selected, e.g., by antibiotic resistance to G418, kanamycin, or hygromycin. In some cases, amplified sequences can be ligated into a mammalian expression vector such as pcDNA3 (Invitrogen) and then transcribed and translated in vitro using wheat germ extract or rabbit reticulocyte lysate.

Polypeptides provided herein can be purified by known chromatographic methods including DEAE ion exchange, gel filtration, and hydroxylapatite chromatography. See, e.g., Van Loon and Weinshilboum, *Drug Metab. Dispos.*, 18:632-638 (1990); and Van Loon et al., *Biochem. Pharmacol.*, 44:775-785 (1992). Polypeptides provided herein can be modified to contain an amino acid sequence that allows the polypeptide to be captured onto an affinity matrix. For example, a tag such as c-myc, hemagglutinin, polyhistidine, or FlagTM (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within a polypeptide including at either the carboxyl or amino terminus. Other fusions that can be useful include enzymes that aid in the detection of a polypeptide, such as alkaline phosphatase. Immunoaffinity chromatography also can be used to purify polypeptides provided herein.

Any suitable method, such as PCR, can be used to obtain an isolated nucleic acid encoding a polypeptide provided herein. The term "nucleic acid" as used herein encompasses both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be the sense strand or the antisense strand. In addition, nucleic acid can be circular or linear.

The term "isolated" as used herein with reference to nucleic acid refers to a naturally-occurring nucleic acid that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one

on the 3' end) in the naturally-occurring genome of the organism from which it is derived. For example, an isolated nucleic acid can be, without limitation, a recombinant DNA molecule of any length, provided one of the nucleic acid sequences normally found immediately flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid sequence.

The term "isolated" as used herein with reference to nucleic acid also includes any non-naturally-occurring nucleic acid since non-naturally-occurring nucleic acid sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome. For example, non-naturally-occurring nucleic acid such as an engineered nucleic acid is considered to be isolated nucleic acid. Engineered nucleic acid can be made using common molecular cloning or chemical nucleic acid synthesis techniques. Isolated non-naturally-occurring nucleic acid can be independent of other sequences, or incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or the genomic DNA of a prokaryote or eukaryote. In addition, a non-naturally-occurring nucleic acid can include a nucleic acid molecule that is part of a hybrid or fusion nucleic acid sequence.

It will be apparent to those of skill in the art that a nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest is not to be considered an isolated nucleic acid.

A nucleic acid provided herein can be at least about ten nucleotides in length. For example, the nucleic acid can be about 10, 11, 15-20 (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length), 20-50, 50-100 or greater than 100 nucleotides in length (e.g., greater than 150, 200, 250, 300, 350, 400, 450, 500, 750, or 1000 nucleotides in length). Nucleic acids provided herein can be in a sense or antisense orientation, can be identical or complementary to the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33, and can be DNA, RNA, or nucleic acid analogs. Nucleic acid analogs can be modified at the base moiety, sugar moiety, or phosphate backbone to improve, for example, stability, hybridization, or solubility of the nucleic acid. Modifications at the base moiety include deoxyuridine for deoxythymidine, and 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine. Modifications of the sugar moiety can include modification of the 2' hydroxyl of the ribose sugar to form 2'-O-methyl or 2'-O-allyl sugars. The deoxyribose phosphate backbone can be modified to produce morpholino nucleic acids, in which each base moiety is linked to a six membered, morpholino ring, or peptide nucleic acids, in which the deoxyphosphate backbone is replaced by a pseudopeptide backbone and the four bases are retained. See, for example, Summerton and Weller, *Antisense Nucleic Acid Drug Dev.*, 7:187-195 (1997); and Hyrup, et al., *Bioorgan. Med. Chem.*, 4:5-23 (1996). In addition, the deoxyphosphate backbone can be replaced with, for example, a phosphorothioate or phosphorodithioate backbone, a phosphoroamidite, or an alkyl phosphotriester backbone.

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Nucleic acids provided herein can hybridize, under hybridization conditions, to the sense or antisense strand of a nucleic acid having the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33. The hybridization conditions can be moderately or highly stringent hybridization conditions.

As used herein, moderately stringent hybridization conditions mean the hybridization is performed at about 42° C. in a hybridization solution containing 25 mM KPO₄ (pH 7.4), 5×SSC, 5×Denhart's solution, 50 µg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/mL probe (about 5×10⁷ cpm/µg), while the washes are performed at about 50° C. with a wash solution containing 2×SSC and 0.1% sodium dodecyl sulfate.

Highly stringent hybridization conditions mean the hybridization is performed at about 42° C. in a hybridization solution containing 25 mM KPO₄ (pH 7.4), 5×SSC, 5×Denhart's solution, 50 µg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/mL probe (about 5×10⁷ cpm/µg), while the washes are performed at about 65° C. with a wash solution containing 0.2×SSC and 0.1% sodium dodecyl sulfate.

Hybridization can be done by Southern or Northern analysis to identify a DNA or RNA sequence, respectively, that hybridizes to a probe. The DNA or RNA to be analyzed can be electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with a probe using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., (1989) Molecular Cloning, second edition, Cold Spring harbor Laboratory, Plainview, N.Y. Typically, a probe is at least about 20 nucleotides in length. For example, a probe corresponding to a 20 nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33 can be used to identify an identical or similar nucleic acid. In addition, probes longer or shorter than 20 nucleotides can be used. A probe can be labeled with a biotin, digoxigenin, an enzyme, or a radioisotope such as ³²P.

Isolated nucleic acids provided herein also can be chemically synthesized, either as a single nucleic acid molecule (e.g., using automated DNA synthesis in the 3' to 5' direction using phosphoramidite technology) or as a series of oligonucleotides. For example, one or more pairs of long oligonucleotides (e.g., >100 nucleotides) can be synthesized that contain the desired sequence, with each pair containing a short segment of complementarity (e.g., about 15 nucleotides) such that a duplex is formed when the oligonucleotide pair is annealed. DNA polymerase is used to extend the oligonucleotides, resulting in a single, double-stranded nucleic acid molecule per oligonucleotide pair, which then can be ligated into a vector.

Antibodies

An antibody that can bind to a polypeptide provided herein can be made and purified using methods known to those skilled in the art (e.g., the methods described herein). For example, an antibody that can bind to a polypeptide provided herein can be affinity purified from the serum of an animal (e.g., a mouse, rat, rabbit, goat, donkey, horse, duck, or chicken) that received a substantially pure polypeptide provided herein under conditions that illicit an immune response to the polypeptide. In some cases, an antibody that can bind to a polypeptide provided herein can be purified from the supernatant of a B cell hybridoma that produces such an antibody.

An antibody that can bind to a polypeptide provided herein can be monoclonal or polyclonal and can be, for example, a single chain Fv, chimeric antibody, or an Fab fragment.

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Fungus-Induced Eosinophil Degranulation

Eosinophils belong to the granulocyte class of white blood cells, and contain cytoplasmic granules that stain with the acidic dye eosin. Eosinophils are the main effectors of antibody-dependent cell-mediated cytotoxicity against multicellular parasites that provoke IgE antibodies. Their role seems to be to engulf and destroy the precipitated antigen-antibody complexes produced in humorally based immune reactions. An elevated eosinophil count usually is seen in allergic reactions, and numerous eosinophils are chemotactically aggregated at sites where antigen-antibody complexes are found.

As used herein, "fungus-induced eosinophil degranulation" refers to eosinophil degranulation in response to one or more antigens from fungal cells (e.g., from fungal cell extracts or fungal culture supernatants). Degranulation is the release of toxic molecules such as eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and MBP that are contained within eosinophil granules; this release typically causes damage to or death of cells in the vicinity of the degranulating eosinophils.

Eosinophil degranulation can be achieved in vitro as described in the example section herein. In some cases, a fungal preparation (e.g., a fungal cell extract or fungal culture supernatant) can be added to an eosinophil to induce degranulation. As used herein, a "fungal cell extract" is a preparation that contains factors (e.g., polypeptides) found within a fungal cell (e.g., in the cytoplasm, membranes, or organelles of a fungal cell). The term "fungal culture supernatant" refers to media obtained from culturing fungal cells. A fungal culture supernatant can be manipulated to form solid material. For example, a fungal culture supernatant can be obtained by removing fungal organisms from a fungal culture. The resulting supernatant then can be concentrated such that any remaining material (e.g., fungal polypeptides) form concentrated liquid or dry material. This dry material can be a fungal culture extract.

A cell extract or culture supernatant from any suitable type of fungus can be used to induce degranulation, including extracts and supernatants from those fungi listed above (e.g., *Alternaria*, *Candida*, *Aspergillus*, or *Cladisporium*). *Alternaria* cell extracts and culture supernatants are particularly useful. These can be obtained by standard laboratory cell culture and extract preparation techniques. Alternatively, fungal cell extracts and culture supernatants are commercially available (e.g., from Greer Laboratories, Lenoir, N.C.). Eosinophils can be obtained by, for example, purification from an individual's blood. Methods for such purification are known in the art.

Eosinophil degranulation can be stimulated in vitro by, for example, incubating a fungal preparation (e.g., a volume of *Alternaria* culture supernatant or 50 µg/mL of an *Alternaria* culture supernatant extract) with an eosinophil (e.g., purified eosinophils). Any incubation time (e.g., 1, 2, 3, 4, 5, 6, 7, or more hours) can be used. For example, an incubation time from about 2 to about 6 hours can be used. Any amount of a fungal preparation can be used. For example, the amount of a fungal extract can range from about 10 µg/mL to about 100 mg/mL (e.g., about 50, 100, 200, 300, or more µg/mL). Degranulation can be measured by a number of methods, including those known in the art. Degranulation can be assessed by, for example, measuring the release of markers such as ECP, EPO, MBP, or EDN. Non-limiting examples of methods for measuring marker levels include protein-based methods such as ELISA assays and western blotting. Alternatively, degranulation can be assessed by visual inspection of eosinophils by microscopy (e.g., using an electron microscope) to detect the presence of empty granules.

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Identifying an Inhibitor of Fungus-Induced Eosinophil Degranulation and/or Inflammation

This document provides methods and materials that can be used to identify an agent that inhibits fungus-induced eosinophil degranulation and/or inflammation. For example, an inhibitor of fungus-induced eosinophil degranulation can be identified by contacting an eosinophil with a polypeptide provided herein in the presence and absence of a test agent, and measuring levels of degranulation (e.g., by measuring EDN output or MBP output, or by observing empty granules within eosinophils viewed by microscopy). A test agent can be identified as an inhibitor of eosinophil degranulation if the level of degranulation is reduced in the presence of the test agent as compared to the level of degranulation observed in the absence of the test agent. By "reduced" is meant that the level of degranulation in the presence of the test agent is less (e.g., 1% less, 5% less, 10% less, 50% less, 90% less, or 100% less) than the level observed without the test agent.

Molecules belonging to any of a number of classes can be used as test agents. For example, molecules that are polypeptides (i.e., amino acid chains of any length, regardless of modification such as phosphorylation or glycosylation), oligonucleotides, esters, lipids, carbohydrates, and steroids can be used as test agents. Molecules that are protease inhibitors may be particularly useful. Such protease inhibitors can be included within a cocktail of inhibitors (e.g., inhibitor cocktails that are commercially available from Roche Molecular Biochemicals, Indianapolis, Ind.) or can be individual protease inhibitors (e.g., a single serine protease inhibitor such as AEBSF).

In some cases, an inhibitor of fungus-induced inflammation can be identified by contacting an animal model (e.g., a mouse model) with a polypeptide provided herein in the presence and absence of a test agent, and measuring levels of inflammation. A test agent can be identified as an inhibitor of inflammation if the level of inflammation is reduced in the presence of the test agent as compared to the level of inflammation observed in the absence of the test agent.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1

The Abnormal Immunologic Response of CRS Patients to Fungal Antigens

The responses of peripheral blood mononuclear cells (PBMC) from CRS patients to fungal antigens were characterized. The cytokine responses from CRS patients and normal volunteers, when stimulated with extracts from four common environmental fungal species—including *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*, were examined. In the Examples section, *Alternaria* refers to *Alternaria alternata* unless specified otherwise. In FIG. 1, PBMC from about 90% of the CRS patients, but not those from normal individuals, produced both IL-5 and IL-13 when exposed to *Alternaria*, *Aspergillus*, or *Cladosporium*, but there were no differences in the amounts of these cytokines between allergic and non-allergic CRS patients. In response to *Alternaria*, PBMC from CRS patients produced about 5-times more IFN- γ than PBMC from normal individuals. Furthermore, levels of serum IgG antibodies to *Alternaria* and *Cladosporium* were increased in CRS patients compared to normal individuals ($p<0.01$), and the increased humoral (serum IgG antibody)

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response strongly correlated with the increased cellular (IL-5 production) response to *Alternaria* ($r=0.619$, $p<0.01$) (FIG. 2). In contrast, <30% of patients had elevated serum levels of IgE antibody to *Alternaria*, and there was no correlation between the serum levels IgE antibody and the cellular response to *Alternaria*. Overall, CRS patients likely exhibit exaggerated humoral and cellular responses, both Th1 and Th2 types, to common airborne fungi, particularly *Alternaria*.

The following was performed to determine why <30% of the CRS patients have IgE antibodies to fungi, while about 90% of them exhibit Th2-like PBMC responses. Production of IgE occurs through sequential switching events from μ to $\gamma 4$ to ϵ . With chronic antigen exposure, IgG4-switched B memory cells are induced, and these IgG4-switched B memory cells may undergo a secondary switch to IgE. FIG. 3 shows that 60% of the patients with CRS had specific IgG4 antibodies to *Alternaria*; 20% of patients with seasonal allergic rhinitis (AR) had anti-*Alternaria* IgG4, and none of the normal individuals did. In contrast, there was no significant difference in the levels of IgG4 antibodies to *Aspergillus* among the three groups. Thus, patients with CRS may have had an increased exposure to *Alternaria*, but not to *Aspergillus*, or they may have had an enhanced "modified Th2 response" to *Alternaria*, or both.

Epithelial cells are likely participants among the important cellular network of immune and inflammatory responses in the airways. It was found that nasal polyp epithelial cells obtained from CRS patients produce large quantities of IL-8 and GM-CSF. Conditioned media containing GM-CSF markedly enhanced activation of blood eosinophils, suggesting that the products of not only lymphocytes, but also epithelial cells activate airway eosinophils in nasal polyps.

Example 2

Eosinophil Activation and Degranulation in CRS

Asthma and CRS coexist clinically in >50% of patients with CRS. Histologic specimens from refractory CRS patients undergoing endoscopic sinus surgery were examined. Specimens from all CRS patients (22/22) revealed epithelial changes including shedding and basement membrane thickening. Striking eosinophilic inflammation, which did not differ between allergic and non-allergic patients, was also detected in all CRS patients. These findings, coupled with the clinical coexistence of both diseases, suggest that the same pathologic disease process is manifest as CRS in the upper airway and as asthma in the lower airway.

Eosinophilic inflammation in CRS patients was characterized using specific immunological probes. Conventionally, Grocott-methenamine silver (GMS) staining can detect fungi in pathologic specimens; however, this technique can be inconsistent because it lacks sensitivity and specificity. Chitinase is an enzyme, which selectively and specifically binds to chitin in fungal cell walls. Fluorescein-labeled chitinase was used and detected one or more fungal hyphae within the sinus mucus of 54/54 (100%) of consecutive surgical patients with CRS. Fungi were in the airway lumen but not within the airway tissues, suggesting that CRS is not an invasive fungal infection. Because PBMC from CRS patients exhibited vigorous cytokine responses to *Alternaria* (FIG. 1), a polyclonal antibody to *Alternaria* was used to investigate the presence of fungi in sinus specimens from CRS patients. Rabbits were immunized with crude *Alternaria* extract, and as expected, this anti-*Alternaria* cross-reacted with other fungi, including *Aspergillus*, *Cladosporium*, and *Penicillium*, but not with

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bacteria. In FIG. 4C, anti-*Alternaria* antibody clearly visualized fungal hyphae and fungal products in the clusters of inflammatory cells (i.e., eosinophils) within the sinus lumen.

To characterize the extent and location of eosinophilic inflammation, antibody to eosinophil major basic protein (MBP) were used. All tissue specimens from CRS patients exhibited intact eosinophils, but diffuse extracellular MBP deposition, as a marker of eosinophil degranulation, was rare. In contrast, all mucus specimens exhibited abundant diffuse extracellular MBP deposition within or around the clusters of eosinophils (FIG. 4D). Thus, release and deposition of the toxic MBP from eosinophils seem to occur mainly within the airway lumen, but not in airway tissues. This observation and the presence of fungal hyphae and fungal products within the airway lumen suggested that the eosinophilic inflammation of CRS may be part of a normal, but clearly exaggerated, immune response to environmental and airborne fungal organisms. The activation mechanisms of eosinophils in vivo in CRS and asthma have been poorly understood.

The following was performed to determine whether human eosinophils have an innate capacity to respond to environmental fungal organisms. Human eosinophils were incubated with extracts from common environmental airborne fungi. As shown in FIG. 5, *Alternaria* and *Penicillium* induced remarkable degranulation (e.g., eosinophil-derived neurotoxin (EDN) release) of eosinophils from normal healthy individuals. No opsonization or sensitization with IgE or IgG antibodies was necessary. *Alternaria* also strongly induced other activation events in eosinophils from healthy individuals, including increases in intracellular calcium concentration ([Ca²⁺]_i), cell surface expression of CD63 and CD11b, and production of IL-8. *Alternaria* did not induce neutrophil activation, suggesting cellular specificity of the *Alternaria* response. The *Alternaria*-induced eosinophil [Ca²⁺]_i response and degranulation was pertussis toxin (PTX)-sensitive. The eosinophil-stimulating activity in *Alternaria* extract was heat-labile, inactivated by heat treatment at 56° C. for 30 minutes, and had a molecular mass about 30-50 kDa (FIG. 6). Thus, eosinophils, but not neutrophils, likely possess G protein-dependent cellular activation machinery that directly responds to an *Alternaria* protein or glycoprotein product(s).

The following was performed to examine whether eosinophils can respond to proteases. Protease-activated receptors (PARs) are a unique class of G protein-coupled seven transmembrane receptors, which are activated by proteolytic cleavage of the amino terminus of the receptor itself (FIG. 7). Four members of this family, including PAR-1, -2, -3, and -4, have been described elsewhere. In the case of PAR-2 (FIG. 7), proteolytic cleavage by a certain protease (e.g., trypsin) exposes its new N-terminus (SLIGKV; SEQ ID NO:38), which binds to the ligand-binding site in the second extracellular loop and results in activation of downstream events. Human eosinophils were found to express PAR-2 constitutively and found to be activated by serine and cysteine proteases, such as trypsin and papain, through this receptor. Eosinophils were also activated by a natural mite allergen protease, Der f1. PAR-2 may serve as an eosinophil receptor to recognize and respond to proteases from allergens, resulting in active release of pro-inflammatory mediators.

Example 3

Test Hypothesis that Fungi Colonized in Paranasal Sinus and Nasal Cavities are Involved in Persistent Eosinophilic Inflammation in CRS

To examine the clinical significance of fungal colonization in CRS, two clinical trials were performed to examine the

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efficacy of anti-fungal agents. It was hypothesized that anti-fungal agents will reduce the fungal burden in the upper airways, resulting in less antigenic stimulation of immune cells, less airway inflammation, and improved clinical outcomes. The first aim was to establish the safety and demonstrate potential clinical efficacy of intranasal antifungal drug therapy in patients with CRS in a pilot trial. This prospective, open-label trial used amphotericin B as a medical treatment in 51 randomly selected CRS patients. The antifungal was applied intranasally using 20 mL of a 100 µg/mL solution twice daily for a mean of 11 months (minimum of 3 months). Using amphotericin B, improvement of sinusitis symptoms was observed in 38/51 (75%) of patients. Endoscopically, 18/51 (35%) patients became disease free and an additional 20/51 (39%) improved by at least one stage. No effect was seen in 13/51 (25%) patients. The available CT scans pre- and post-treatment (n=12) demonstrated a significant reduction in the inflammatory mucosal thickening. Thus, this open-label pilot trial demonstrated that direct muco-administration of an antifungal drug is both safe and potentially effective to treat patients with CRS.

Second, to address the efficacy of intranasal antifungal agents more objectively, a randomized, placebo-controlled, double-blind, single center trial was performed to treat 30 randomly selected CRS patients. Patients instilled 20 mL amphotericin B (250 µg/mL) or placebo to each nostril twice daily for 6 months. Twenty-four patients completed the 6 months of treatment. Patients receiving amphotericin B showed reduced mucosal thickening on CT scans compared to placebo ($p=0.030$). Between group comparisons of the changes in the intranasal mucus levels of EDN, as a marker of eosinophilic inflammation, showed a reduction in the amphotericin B group and an increase in the placebo group ($p=0.046$). The changes in the endoscopic scores improved in the amphotericin B group compared to placebo ($p=0.038$). While the group comparison showed statistically significant differences, careful examination of individual patient data in the amphotericin B group showed a spectrum of efficacy. Some patients responded well to the treatment, but others not as well. Thus, fungi may be important in the development of CRS in certain patients.

Example 4

Mechanisms and Molecules Involved in Eosinophil Degranulation in Response to *Alternaria*

The majority of previous studies in anti-fungal immune responses used the following models: animal infection in in vivo systems (e.g., *Candida albicans*, *Aspergillus fumigatus*), or entire fungal hyphae or conidia (e.g., *C. albicans*, *A. fumigatus*), a yeast model (e.g., zymosan), and isolated fungal carbohydrate macromolecules (e.g., β-glucan, mannan) in in vitro systems. These studies pointed to roles for TLRs, in particular TLR2 and TLR4, and to other pattern recognition receptors that immune cells, such as macrophages and neutrophils, use to recognize fungi. Because eosinophils express little TLR2 or TLR4 and the active component(s) in *Alternaria* extract was a heat-labile molecule(s) with an approximate 30-50 kDa molecular mass (FIG. 6), it was speculated that an *Alternaria*-derived protease(s) (not carbohydrates), interacting with eosinophil PAR-2, may be involved in the eosinophils' responses to *Alternaria*. Since no specific small molecule inhibitor for PAR-2 is available, a desensitization approach was used. As shown in FIG. 8, pre-incubation of eosinophils with the PAR-2 agonistic peptide, SLIGKV (SEQ ID NO:38), significantly inhibited the eosinophils' calcium

response to *Alternaria* extract. Similarly, an N-terminal reversed peptide (LSIGKV; SEQ ID NO:35), which is known to inhibit activation of PAR-2, also inhibited the eosinophils' calcium response to *Alternaria*; a control scramble peptide (GLIVKS; SEQ ID NO:36) showed no effects. Eosinophil degranulation induced by *Alternaria* extract was also significantly and specifically inhibited by the LSIGKV (SEQ ID NO:35) peptide (FIG. 8, panel B). In contrast, degranulation induced by PAF or PMA was not affected by the LSIGKV (SEQ ID NP:35) peptide. Thus, PAR-2 is likely involved in the eosinophils' calcium and degranulation responses to *Alternaria* extract.

A search through a current database of known *Alternaria* allergens did not reveal any relevant proteases. A fluorescent quenched peptide substrate (Abz-SKGRSLIGK(Dnp)D) (SEQ ID NO:37), which spans the trypsin-cleavage site (between R and S) of PAR-2 was synthesized, and used it in an in vitro assay for PAR-2 cleavage and activation. As shown in FIG. 9, trypsin, as positive control, clearly cleaved this peptide, and a serine protease inhibitor, APMSF, inhibited the activity. *Alternaria* extract also potentially cleaved this peptide, but it was insensitive to APMSF. *Alternaria*'s activity was abolished when aspartate protease(s) was removed from the extract by pepstatin A agarose (FIG. 9); pepstatin A is a highly specific inhibitor for aspartate protease. Furthermore, eosinophil degranulation induced by *Alternaria* extract was significantly inhibited by pepstatin A agarose, but not by control agarose or APMSF. Thus, an aspartate protease(s) in *Alternaria* extract, but not a serine protease(s), may be involved in the activation of eosinophils through PAR-2. This observation was confirmed by using other aspartate protease inhibitors, including alkalo-thermophilic *bacillus* inhibitor (ATBI), nelfinavir, and ritonavir.

Eosinophils may be the only cell that can recognize *Alternaria*. In FIG. 10, an airway epithelial cell line, BEAS-2B, produced and released IL-6 when incubated with *Alternaria* extract for 24 hours. Extracts of *Aspergillus*, *Candida*, and *Penicillium*, did not induce IL-6 production; rather, both *Aspergillus* and *Penicillium* inhibited the baseline production of IL-6. BEAS-2B stimulated with *Alternaria* also produced other pro-inflammatory factors such as IL-8 and GM-CSF. This *Alternaria*-induced IL-6 production was inhibited by ATBI, nelfinavir, ritonavir or pepstatin A-agarose treatment of *Alternaria* extract by about 60% to 90%; ritonavir results are shown in FIG. 11. In contrast, TNF- α -induced IL-6 production was not affected by these treatments. Furthermore, a peptide antagonist for PAR-2, LSIGKV (SEQ ID NO:35), partially (~40%) but significantly inhibited *Alternaria*-induced IL-6 production by BEAS-2B cells. Thus, through its aspartate protease activity, *Alternaria* may activate airway epithelial cells; this activation is partially mediated by PAR-2.

A series of efforts have been initiated to identify and isolate protease(s) from *Alternaria*. A preliminary biochemical characterization showed that, at pH 7.5, the *Alternaria* activity towards eosinophils binds to hydroxyapatite, DEAE Sepharose, and phenyl-Sepharose, but not to a variety of cation exchange or lectin columns. In FIG. 12, DEAE fractionation of an *Alternaria* extract showed a single peak with strong aspartate protease activity, as detected by a malaria aspartate protease substrate. The peak of aspartate protease activity coincided with the peak of the PAR-2 cleavage activity, and the aspartate protease activity paralleled each fraction's ability to induce eosinophil degranulation.

Partial characterization of *Alternaria* extract. Three strategies were used to begin characterizing the *Alternaria* products involved in eosinophil degranulation. First, the *Alternaria* extract was subjected to membrane filtration. After

filtration with a YM100 Centricon® membrane, the filtrate stimulated eosinophil degranulation, but the retentate did not. After filtration with a YM10 Centricon® membrane, the retentate stimulated eosinophils, but the filtrate did not. Thus, the eosinophil-stimulatory activity in the *Alternaria* extract is likely between 10 and 100 kDa. Second, *Alternaria* extracts, which had been treated at 56° C. or 100° C. for 30 min, did not induce EDN release (FIG. 25A), but extracts treated at 4° C. or 37° C. for 30 min did induce EDN release, suggesting that it is a heat-labile protein(s) or glycoprotein(s). The activity of a cytokine, IL-5, to induce EDN release was abolished by treatment at 100° C., but not by treatment at 56° C. or lower temperatures. Third, size exclusion chromatography was used (FIG. 25B), and the column fractions tested for their abilities to induce eosinophil degranulation. Although the absorbance profile shows a broad peak from fractions 32 through 37, the most potent eosinophil degranulation activity appeared in fraction 32 with a molecular mass about 60 kDa.

PBMCs obtained from a CRS patient were incubated with fractions 30 or 32, and the level of cytokine production was measured (FIGS. 44 and 45).

Polypeptides (e.g., enzymes) implicated in the activation of eosinophils and promotion of eosinophilic inflammation in a murine model were identified. Proteins in HPLC DEAE fraction #18 and the eluate from pepstatin A agarose were trypsin digested, and the resulting peptides were subjected to nLC-microESI-MS/MS analysis using a Finnigan LTQ system (Thermo Electron Corporation, Waltham, Mass.). Peptide mass fingerprinting with SEQUEST software (distributed by Thermo Electron Corporation, Waltham, Mass.) was used to identify peptides existing in these fractions using the resulting peptide mass data and a database of predicted *Alternaria brassicicola* proteins derived from expressed sequence tags (ESTs) and the *A. brassicicola* whole genome shotgun sequence information. SEQUEST correlates uninterpreted tandem mass spectra of peptides with amino acid sequences from protein and nucleotide databases. SEQUEST will determine the amino acid sequence of the peptide fragments, and thus the full length protein(s) can be identified. Proteins in the database were predicted using ab initio gene finding and protein prediction software FgeneSH (Softberry, Inc., Mount Kisco, N.Y.). SEQUEST is a registered trademark of the University of Washington. SEQUEST uses algorithms described in U.S. Pat. Nos. 6,017,693 and 5,538,897.

The fungal genes encoding these immunostimulatory proteins were identified using the above described approach. The implicated immunostimulatory proteins identified in these fractions were then further annotated by BlastP analysis against the GenbankNR database and the MEROPS peptidase database. The MEROPS database is an information resource for peptidases (also termed proteases, proteinases and proteolytic enzymes) and the proteins that inhibit them and was developed and web accessible at the Sanger Institute, UK. Furthermore, all candidate proteins were subjected to InterPro analysis. InterPro is a database of protein families, domains and functional sites in which identifiable features found in known proteins can be applied to unknown protein sequences. InterPro analysis is web accessible and a public service available at the European Bioinformatics Institute (EMBL-EBI). The annotated proteins include several proteases belonging to S53 and M38 families, several predicted glycolytic enzymes, superoxide dismutase, a ribosomal protein, S-adenosyl-homocysteine lyase, and several others (Table 1).

TABLE 1

Identified polypeptides.	
SEQ ID NO:	Functional Annotation
2	<i>Alternaria alternata</i> endoxylanase - gil6179886/gbl/AF176570.1 AF176570
4	S-adenosyl-L-homocysteine hydrolase
6	glycosyl hydrolase family 61 (Endo-1,4-beta-glucanase IV/ Cellulase IV)
8	glycosyl hydrolase family 31, alpha-glucosidase
10	peptidase family S53 contains acid-acting endopeptidases
12	peptidase family S53 contains acid-acting endopeptidases
14	contains predicted signal peptide for secretion
16	<i>A. alternata</i> 60S acidic ribosomal protein P1 (Allergen Alt a12) P49148 GI:1350779
18	Superoxide dismutase
20	contains predicted transmembrane regions
22	Peptidase family M38 (beta-aspartyl dipeptidase family)
24	contains predicted signal peptide and transmembrane domains
26	Unknown
28	Arginase
30	glycosyl hydrolase family 7 Exoglucanase 1 precursor (Exoglucanase I) (Exocellobiohydrolase I) (1,4-beta-cellulobiohydrolase I) (Beta-glucancellobiohydrolase I)
32	glycosyl hydrolase family 6 - cellobiohydrolase II cellobiose dehydrogenase

The *Alternaria brassicicola* nucleic acid sequence for each identified *Alternaria alternata* candidate along with the predicted *Alternaria brassicicola* amino acid sequence is set forth in FIGS. 27-39.

Example 5

Production of Immunostimulatory Molecules by Live *Alternaria*

Spores of *A. alternata* were obtained, and the effects of the fungus itself on eosinophil activation were examined. Various numbers of spores were suspended in RPMI medium with 10% FCS and incubated in tissue culture wells for 12 hours to induce germination. A fixed number of isolated human eosinophils were added to the wells and incubated for an additional 4 hours. These eosinophils showed strong conjugate formation with the germinating *Alternaria* fungal spores (FIG. 13A). Furthermore, these eosinophils became activated and released their granule proteins into the supernatants (FIG. 13B). To characterize the growth pattern and production of immunostimulatory molecules by *Alternaria* further, GFP-transformed *A. alternata* were used (FIG. 14). Currently, there is no standardized scientific method to quantitate fungal growth. However, these transformed fungi have a technical advantage; fungal growth can be quantitated by measuring the fluorescence intensity using a plate reader or spectrophotometer. Production of so-called "allergens" by fungi can be significantly increased during and after their germination. FIG. 15B shows that the PAR-2-stimulating enzymatic activity(ies) is clearly produced by *A. alternata* during their germination and hyphal growth. The growth of fungi (FIG. 15A) and production of PAR-2 activating enzymes (FIG. 15B) dramatically increased when fungi were incubated in the presence of airway mucin. Thus, *A. alternata* likely produces PAR-activating enzyme(s) during their germination and growth, in particular when they germinate on mucosal surfaces, and eosinophils demonstrate a vigorous inflammatory response against these germinating fungi. The model of a spore/eosinophil mixed culture provides a tool to dissect the role of specific *Alternaria* molecule(s) in the eosinophil's recognition of and response to this fungus.

The polypeptide having the amino acid sequence set forth in SEQ ID NO:2 was recombinantly produced in *E. coli* and tested for the ability to stimulate eosinophil degranulation. This polypeptide stimulated eosinophil degranulation, as measured by EDN release, in a concentration-dependent manner.

Example 6

In Vivo Mouse Model of Immune Response to *Alternaria*

In FIG. 1, PBMC from CRS patients show increased cellular and humoral immune responses to *Alternaria*. To dissect the role of immune cells in their responses to fungi, a mouse model was developed. Because CRS patients showed an increased immune response to fungi, BALB/c mice were sensitized to *Alternaria* by intraperitoneal (i.p.) injection of *Alternaria* extract (Greer Laboratories) and subsequently challenged mice intranasally (i.n.) with the same extract. Mice sensitized and challenged with *Alternaria* exhibited striking airway eosinophilia. Airway eosinophilia was also observed in mice sensitized with PBS (no antigen) and challenged intranasally with *Alternaria*. Thus, mice might have an innate ability to produce an airway eosinophilic response to certain fungi, which may be similar to the innate Th2 and eosinophilic responses to helminth parasites in mice.

To test this hypothesis, fungal extracts or OVA (as a control) were administered intranasally to naive mice without prior sensitization on days 0, 3, and 6, and airway inflammation was analyzed on day 8. Mice exposed to culture supernatant or cellular extract of *Alternaria* exhibited significant airway eosinophilia (FIG. 16). *Aspergillus* induced mild airway eosinophilia. In contrast, *Candida* induced neutrophilia, but no eosinophilia. This airway eosinophilia in *Alternaria*-exposed mice is probably not due to accidental prior sensitization of the animals to *Alternaria* for the following reasons: 1) mice from different animal vendors showed similar eosinophilic responses; 2) no IgG or IgE antibodies to *Alternaria* were detected in naive mouse serum; and 3) spleen cells from naive mice cultured with *Alternaria* antigen did not produce IL-4 or IL-5. In addition, the airway eosinophilic response to *Alternaria* was reproducible among different strains of mice including BALB/c, C57BL/6, C3H/HeJ, C3H/HeSnJ, and WBB6F1/J-KitW/KitW-v.

Generally, an intact adaptive immune system, especially the Th2 cells, is needed to develop robust airway eosinophilia in mice sensitized and challenged with OVA as described elsewhere. The contributions of the adaptive immune system in the development of airway eosinophilia in naive *Alternaria*-exposed mice were investigated. In FIG. 17A, there were no differences in the early eosinophilia (i.e., days 0.5 and 5) between wild-type animals and Rag-1^{-/-} mice, suggesting that an innate immune response mediated the early eosinophilic response to *Alternaria*. In contrast, an adaptive immune system, presumably T cells, was required for further development of eosinophilia at a later time point (i.e., day 8). When *Alternaria* was administered only once to the mouse airways, IL-5 and IFN- γ , but not IL-4, were detected in BAL fluids by as early as 3 hours and peaked at 12 hours, suggesting that the early cytokine production does not reflect a typical Th2 pattern. Furthermore, the early IL-5 and IFN- γ responses (12 hours after first exposure) were not reduced in Rag-1^{-/-} mice (FIG. 17B). Rather, IL-5 production was enhanced in Rag-1^{-/-} mice, suggesting that innate immune cells are respon-

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sible for this early production of IL-5 and IFN- γ and that adaptive immune cells may show inhibitory effects on this innate response.

Various molecules and their receptors can be involved in this Th2-like airway inflammation in naïve mice exposed to *Alternaria* in vivo (FIG. 16). In mice, a small amount of LPS interacting with TLR4 is a factor in promoting Th2 sensitization to protein antigens as described elsewhere. In addition, the cysteine proteinase gene from *Leishmania mexicana* has been implicated in the upregulation of Th2 immunity and the downregulation of Th1 immunity to this pathogen in mice. The *Alternaria* preparation contained a minimal amount of LPS (0.4 ng/mg dry weight); thus, each mouse received 0.1 ng of LPS/challenge. Because this amount of LPS is much smaller than that used previously to promote an airway Th2 response to OVA (i.e., 100 ng/challenge, 74), it is very unlikely that LPS contributes to this model. Also, prior treatment of mouse airways with 1 μ g LPS significantly inhibited this early IL-5 production (FIG. 18A). This early IL-5 production was significantly enhanced in mice deficient in TLR-4 (C3H/HeJ) compared to control mice (C3H/HeOuJ) (FIG. 18B). Early IL-5 production was also increased in IL-10 deficient mice compared to wild-type controls (19.1 \pm 8.0 vs 7.6 \pm 2.8, n=4), suggesting a role for IL-10 to down-regulate the early IL-5 response. Altogether, naïve mice likely show innate IL-5 and eosinophilic responses to airway exposure of *Alternaria*, and this innate response may be down-regulated by activation of TLR-4 or by production of IL-10.

The in vitro experiments suggested a potential role for *Alternaria* aspartate protease(s) in the activation of eosinophils (FIG. 9) and airway epithelial cells (FIG. 11). Thus, it is hypothesized that the protease(s) similar to those involved in eosinophil degranulation and airway epithelial cell production of IL-8 in vitro may be involved in the development of airway eosinophilia in vivo in mice. To address this question in vivo, *Alternaria* extract was treated with pepstatin A-agarose to remove aspartate protease(s) or control agarose (FIG. 9) and was administered to naïve mice. Pepstatin A treatment significantly inhibited both early production of IL-5 at 12 hours and airway eosinophilia on day 8 (FIG. 19). FIG. 20 shows that the same peak fraction from the DEAE fractionation (i.e., Fraction #18 of FIG. 12), which contained strong aspartate protease activity and potently induced eosinophil degranulation, also induced marked airway eosinophilia when administered into naïve mice.

Example 7

Effects of Glycolytic Enzyme Homologs on Immune Cell Activation In Vitro and In Vivo

The following was performed to characterize the responses of eosinophils (in vitro) and mouse airways (in vivo) to the homologous enzymes from other fungal species, some of which are commercially available. In Table 1, *A. alternate* xylanase (a glycolytic enzyme) (AAF05698.1) was identified by pepstatin A-affinity chromatography of an *Alternaria* extract. Thus, the commercially available xylanase isolated from *Trichoderma viride* was used (Sigma catalog# X3876), and its biological activity examined. Incubation of isolated human eosinophils with *Trichoderma* xylanase induced EDN release (FIG. 21A). Instillation of *Trichoderma* xylanase into the airways of naïve mice induced increases in airway levels of IL-5 in vivo (FIG. 21B); IL-5 production was not inhibited in Rag-1 $-/-$ mice. Thus, the fungus-derived immunostimulatory activities are not limited to *Alternaria*, but are likely

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shared with certain other fungal species. Furthermore, the eosinophil activation assay in vitro and the mouse airway response in vivo, as well as the airway epithelial cell culture provide models to examine the effects of specific immunostimulatory molecules produced by fungi and to dissect the molecular mechanisms involved in this fungus-immune cell interaction.

Example 8

Characterizing the Airway Immune and Inflammatory Responses to Environmental Fungi in Patients with CRS

PBMC are isolated from CRS patients with or without nasal polyps, AR patients and normal individuals, and their proliferative and cytokine responses to fungal antigens are compared. CD4+ cell proliferation is measured by dilution of the carboxyfluorescein diacetate succinimidyl ester (CFSE). Twenty-five cytokines and chemokines in the supernatants are quantitated simultaneously by a Lumines system.

Stimulated PBMC are stained with antibodies for cell surface markers and intracellular cytokines, and are analyzed by FACS to identify cells producing IL-5, IL-13, and IFN- γ . Special attention is focused on whether CD4+ T cells and CD56+ NK cells produce these cytokines.

Subjects. Patients with CRS are studied, using patients with AR and normal individuals as controls. Patients who received systemic glucocorticoids during the past 4 weeks, who are smokers, or who were diagnosed with an immunodeficiency or cystic fibrosis are excluded. The diagnosis of CRS is made based on the fulfillment of all three criteria: i) 2 or more of the following symptoms for more than 12 weeks— anterior or posterior mucopurulent drainage, nasal obstruction, facial pain-pressure-fullness, and decreased sense of smell; ii) anterior rhinoscopy or nasal endoscopy to document signs of inflammation; and iii) sinus CT scan demonstrating isolated or diffuse mucosal thickening. CRS with nasal polyps (CRSwNP) is defined as those CRS patients who now have or who had nasal polyps in the middle meatus, as determined by anterior rhinoscopy or nasal endoscopy. CRS without nasal polyps (CRSSNP) is defined as CRS patients who fulfill all three criteria for CRS as described above, but who do not have demonstrable nasal polyps in the middle meatus both in the past and at present.

Seasonal allergic rhinitis (AR) to ragweed. The clinical diagnosis of AR is established by history, where patients describe the typical seasonal signs of nose itching, sneezing and clear rhinorrhea, and is confirmed with a positive skin test and/or elevated specific serum IgE level for short ragweed antigen. Patients with AR are to have no history or symptoms of CRS or asthma and are to have normal lung function.

Normal Controls. The normal controls are healthy individuals with no history of allergy or asthma and negative skin prick test results to fungi and common aeroallergens.

Demographic Characterization of Patients and Normal Individuals.

Questionnaire: Each patient is asked to complete the questionnaire regarding the history of his or her sinus symptoms, aspirin sensitivity, sinus operations, and recently used and current medications. Patients are also asked regarding their history of asthma and AR, smoking habits, and use of allergen immunotherapy.

Skin tests: Skin prick tests are performed with a battery of 18 commercially available fungal extracts and 8 common aeroallergen extracts, including *Dermatophagoides pteron-*

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yssinus, *D. fariniae*, cockroach, short ragweed pollen, mixed grass pollen, mixed tree pollen, cat epithelium, and dog dander.

Total and specific IgE: Total serum IgE is measured by two-site ELISA. Allergen-specific IgE antibody levels are determined by RAST using 8 fungal allergens and 8 common Aeroallergens.

Assessment of CRS: To assess the extent of the CRS, symptoms and quality of life (QOL) are scored according to the Symptom Score (0-10 visual analogue scale of 6 sinusitis-related symptoms and Gliklich and Metson QOL Score). Sinus CT scans are scored according to CT scoring systems described elsewhere (e.g., the Lund-Mackay staging system and the digital analysis of scanned images).

Sample Size

Given the conservative assumption that IL-5 is produced by PBMC from $\geq 83\%$ of the patients with CRS and is produced in 36% of the normal controls, we are to have 80% power with a probability of a type I error rate of 0.05 with 20 patients in each group. Therefore, 20 CRSwNP, 20 CRSsNP, 20 AR, and 20 normal controls are recruited.

Cell Proliferation and Cytokine Production by PBMC

PBMC are cultured for 24 hours or 96 hours (for cytokine assay) or for 168 hours (for proliferation assay) with or without 25 $\mu\text{g}/\text{mL}$ extracts of *Alternaria*, *Aspergillus*, *Cladosporium*, and short ragweed (Greer Laboratories), 2 $\mu\text{g}/\text{mL}$ tetanus toxoid, or 5 $\mu\text{g}/\text{mL}$ Con-A. The optimal concentrations of antigens and duration of culture have been determined elsewhere. The concentrations of a panel of 25 cytokines and chemokines (IL-1 β , IL-Ra, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, TNF- α , IFN- α , IFN- γ , GM-CSF, MIP-1 α , MIP- β , IP-10, MIG, eotaxin, RANTES, MCP-1) are measured by a Luminex 100 IS system (Upstate) and 25-plex antibody bead kit (Bio-Source International). The differences in the amounts of individual cytokine/chemokines among the groups are analyzed by Mann-Whitney U test. The pattern and cluster of cytokine production in each subject group are analyzed by Spotfire DecisionSite software (Somerville). For the CD4+ T cell proliferation assay, PBMC are labeled with 5 mM CFSE for 10 min before addition of antigens. After culture, PBMC are stained with PE-conjugated anti-CD4 and analyzed by FACS; CFSE dye is diluted in the proliferating population of the CD4+ T cells, and the numbers of cells that have proliferated per 1,000 CD4+ T cells are determined.

A pilot study showed that when PBMCs from a CRS patient were stimulated with *Alternaria* extract, a population of CFSElow CD4+ T cells emerged by day 4, and represented 66.9% of total CD4+ T cells on day 7 (FIG. 22); no changes were observed in PBMCs cultured in medium alone. A side-by-side comparison of a normal individual and a CRS patient in a separate experiment (compared on day 7) showed that a higher proportion of CD4+ cells were CFSElow in the CRS patient than those in the normal individual (43.2% vs. 4.8%) (FIG. 23). In contrast, many CD4+ cells were CFSElow in both the CRS patient and normal individual when they were stimulated with tetanus toxoid (43.2% vs. 47.9%).

FACS Analyses of Cytokine Producing Cells

The PBMCs producing IL-5, IL-13 and IFN- γ are analyzed by FACS. IL-5 is likely produced by CD4+ T cells, CD8+ T cells, and CD56+NK cells. Thus, FITC-conjugated antibodies are used for these cell surface markers and PE-conjugated antibodies to IL-4, IL-5, IL-13, and IFN- γ to identify cytokine-producing cells. After stimulation with antigens, PBMC are re-stimulated with ionomycin plus PMA in the presence of brefeldin A. Cell surface antigens are stained with FITC-conjugated anti-CD3, CD4, CD8 or CD56 (Becton Dickin-

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son). After washing, cells are fixed and permeabilized simultaneously by Cytofix/Cytoperm solution (Pharmingen), and stained with PE-conjugated anti-cytokine or control mouse Ig.

5 In Vitro Organ Culture of Sinus Tissue Specimens from CRS Patients Produce Distinctive Pro-Inflammatory Cytokines

Large quantities of sinus tissue specimens are obtained from CRS patients during endoscopic sinus surgery. Specimens from the ethmoid sinuses of normal individuals (non-allergic, no asthma, no CRS) undergoing septoplasty procedures are used as a negative control. Other disease control specimens are obtained from patients with AR, who undergo septoplasty.

10 To examine the immunological responses by sinus mucosa to fungi, an organ culture system is used, rather than isolated mononuclear cells. Organ culture can allow for the study the mucosal immune responses and tolerance that are likely be mediated by a complex network of epithelial cells, antigen presenting cells, lymphocytes and potentially other mucosal resident cells, and each cellular component may play a role. Tissues are minced into 5-mm pieces, and then cultured with 15 fungal extracts (e.g., *Alternaria*, *Cladosporium*, *Aspergillus*), Con-A or tetanus toxoid for 24 hours or 96 hours. First, the 20 concentrations of 25 cytokines and chemokines, including IL-10, in the supernatants are analyzed by a Luminex system. The concentration of TGF- β is measured by ELISA. Second, once several cytokines (e.g. IL-5) are verified to be produced at elevated levels during the CRS organ culture, the cell types 25 that produce these cytokines are identified. After antigenic 30 stimulation for 96 hours, the tissue specimens are treated with a cocktail of highly pure collagenases (Blendzyme 3, Roche). In preliminary studies, the yield was 12 to 70×10^6 cells/specimen, and the viability was 65~95%. The single cell 35 suspension are recovered after passing through a nylon mesh with 100 μm pore size. The cell types (CD4+, CD8+, CD56+) producing cytokines (IL-5, IL-13, IFN- γ) are analyzed by intracellular cytokine staining and FACS analysis.

40 Subjects. Patients with CRS, who are undergoing endoscopic sinus surgery, are studied, using normal individuals as controls. The criteria for CRS patients and normal individuals are the same as described above. The patients with CRSwNP are enrolled because the patients with CRSwNP tend to have more expensive disease than those with CRSsNP. For this 45 study, patients who are not using nasal or inhaled steroids for 4 weeks before the surgery are specifically selected. The goal is to detect at least 1.5 SD differences in means between two groups as significant with 80% power with a probability of a 50 type I error rate of 0.05. Therefore, tissues from 7 CRS patients and 7 normal controls for each of the 3 experiments are obtained. Because the sample size is not based on preliminary data, a second power calculation is performed once 7 subjects in each group have completed the study. If there is a risk for type II error, the sample size is increased.

55 Analyses of the functions of CD4+CD25+ regulatory T cells. CD4+ T cells are isolated from single cell suspensions of sinus tissue fragments by negative immunomagnetic selection, followed by positive selection for CD25+ cells by magnetic cell sorting (StemCell Technologies). Isolated CD4+ 60 CD25- cells are incubated with serial dilutions of isolated CD4+CD25+ cells in the presence of autologous irradiated mononuclear cells for 96 hours and in the presence or absence of fungal extract (e.g. *Alternaria*). The production of cytokines (IL-5, IL-13, IFN- γ) in the supernatant is measured by 65 ELISA, and the proliferation of CFSE-labeled CD4+CD25- cells is examined. In some experiments, antibodies to IL-10 and IL-10R α -chain and a soluble TGF- β RII-Fc chimeric pro-

tein (all from R&D systems) are included in the culture to examine the role of IL-10 and TGF- β to dampen the cytokine and proliferative responses.

In Vivo Intranasal Challenge with Alternaria in CRS Patients

Subjects. CRS patients without demonstrable IgE antibodies to *Alternaria* are studied using CRS patients with IgE antibodies to *Alternaria* and normal individuals as controls. The criteria for CRS patients and normal individuals are the same as described above, and patients who are not on nasal or inhaled steroids for 4 weeks before the study are selected. The presence or absence of IgE antibodies to *Alternaria* is examined by both skin tests and IgE RAST. About 30% of patients with CRS have demonstrable IgE antibodies to *Alternaria*. Asthma is not required for inclusion; if CRS patients do have a history of asthma, they may be included in the study if their asthma is mild as defined by all of the following parameters; (1) a baseline FEV1 of more than or equal to 80% of predicted, (2) no need for any maintenance therapy for asthma with inhaled steroids, long-acting bronchodilators, or systemic steroids, (3) no need for treatment with theophylline or leukotriene inhibitors on daily basis, and (4) no history of emergency room visits or hospitalization because of asthma in the last ten years. Based on preliminary data, for a dichotomous endpoint (e.g., detectable level of IL-5), a sample-size of n=10 per group provides statistical power of 84% to detect a difference between groups. Statistical power is increased when data are analyzed as continuous variables. 10 subjects are recruited for each of the 3 groups.

Intranasal challenge and sample collection. Intranasal challenge with *Alternaria* is performed as described elsewhere. Briefly, before nasal challenge, CRS patients with IgE antibodies to *Alternaria* undergo endpoint titration to establish the optimal dose for starting their intranasal challenge. Endpoint titration is performed by a skin prick test with escalating or decreasing dosages of *Alternaria* extract (ALK Abello, product#ALTE21P41L) starting at 18 PNU/mL. If there is no reaction (wheal and flare) at 18 PNU/mL, the next higher concentration is tested until a wheal and flare response occurs. If there is a reaction at 18 PNU/mL, the next lowest concentration is tested until no wheal and flare develops. The starting dosage for the nasal challenge for CRS patients with anti-*Alternaria* IgE antibody is the highest concentration that causes no wheal and flare response. CRS patients who do not have IgE antibody to *Alternaria* (i.e., both skin test negative and RAST negative) or normal individuals are started at 18 PNU/mL. For nasal challenge, the *Alternaria* extract (ALK Abello, product# ALTE21P41L) is administered by a metered nasal spray pump (Callipot) that delivers 0.1 mL of extract per nostril. If no reaction occurs, it is proceed with a 3-fold higher concentration (e.g. 54 PNU/mL) up to 40,000 PNU/mL. The interval between each challenge is 15 minutes. The cumulative dose of *Alternaria* received by each subject is <12,000 PNU. The nasal lavage specimens are collected before and 24 hours after the challenge. Three milliliters of saline are introduced into each nostril, and secretions are collected into a container. The specimens are processed immediately for cell count and differentials, and supernatants are stored for cytokine and eosinophil granule protein assays. The peak expiratory flow rate (PEFR) is measured at baseline and after each dose. A pulmonary function test (flow volume loop) is performed with measurement of forced expiratory volume 1 (FEV1) before, immediately after, and 24 hours after the escalating intranasal challenge protocol. There is a stopping rule in place. At baseline and after each challenge, all subjects are asked for their symptoms. These symptoms (nasal blockage, nasal discharge, number of sneezes, nasal itching, difficulty breathing, cough or wheezing) are recorded on a four-

point scale (0 to 3). The total symptom score is calculated as the sum of the individual symptom scores. The nasal challenge is stopped at the dosage of *Alternaria* extract that produces either: i) 1 mL of nasal secretions or more than 5 sneezes within 15 minutes, ii) a symptom score of 3 for two or more of the symptoms mentioned above, or iii) difficulty breathing with a decrease of the PEFR or FEV1 by 15% or more.

Samples and data obtained. Nasal lavage fluids are collected from study subjects before and 24 hours after intranasal challenge, and the total leukocyte counts and differentials are determined. The concentrations of cytokines/chemokines, including IL-4, IL-5, IL-13, IFN- γ , TNF- α , IL-10, and eotaxin, in nasal lavage fluids are quantitated by specific ELISA (Endogen). The sensitivity of these ELISA is generally <0.7 pg/mL. Eosinophil granule MBP and EDN are analyzed by RIA to monitor eosinophilic inflammation.

Example 9

Identifying *Alternaria* Products that Trigger Profound Th2-Like Inflammation In Vitro in Human Airway Cells and In Vivo in Mouse Airways

The following describes methods and materials for producing recombinant candidate *A. alternata* immunomodulatory proteins and characterizing their immune responses in vitro and in vivo. Purified recombinant forms of the *Alternaria* protein candidates are produced. These proteins are used to perform various in vitro and in vivo immunological assays and to elucidate the role of these proteins individually and in concert in CRS pathogenesis.

Candidate proteins identified in Table 1 are expressed recombinantly. Constructs are made to consist of the following: 1) the trpC and ToxA promoter, 2) a PCR amplified cDNA or genomic region from *A. alternata* corresponding to the full-length candidate genes of the enzymes, and 3) a PCR generated histidine tag (e.g., 6x-His) engineered just prior to the stop codon (C-terminus) to aid in purification. These constructs are then introduced into *A. alternata* protoplasts using standard polyethylene glycol (PEG)-mediated fungal transformation approaches. Individual mutants are grown in potato dextrose broth with hygromycin, and expression levels of the introduced genes are verified using RT-PCR or northern blotting, and SDS-PAGE. Individual mutants exhibiting high-level expression of the protein of interest are grown in larger amounts, culture filtrates are purified, and Immobilized Metal Affinity Chromatography (IMAC) for the histidine-tagged protein purification involves using a HPLC system and Ni Sepharose chromatography.

Alternatively, routine recombinant protein expression systems with organisms like *E. coli* and *Pichia pastoris* are used. For example, *E. coli* was used to produce one of eight candidates, *A. alternata* xylanase (AAF05698.1) (FIG. 26).

55 In Vitro and In Vivo Assays for Activity of Recombinant *Alternaria* Proteins

Eosinophil [Ca $^{2+}$]i response and degranulation. For degranulation, isolated eosinophils are incubated with different concentrations of recombinant proteins (10 ng/mL-1 mg/mL) for 3 hours, and EDN released into supernatants is measured by RIA to indicate degranulation. Changes in [Ca $^{2+}$]i are measured using FACS analysis and eosinophils loaded with a calcium indicator, indo-1. The involvement of PAR-2 and proteolytic/glycolytic enzymes is verified by a PAR-2 peptide antagonist, LSIGKV (SEQ ID NO:35), and enzyme inhibitors, such as pepstatin A-agarose, ATBI, ritonavir, and allosamidine. The active cleavage of PAR-2 is

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verified by fluorescent quenched peptide substrate [Abz-SKGRSLIGK(Dnp)D] (SEQ ID NO:37) and by analysis of stimulated eosinophils by FACS and immunoblot using anti-PAR-2 antibody (which recognizes the N-terminus of PAR-2).

Although unlikely, the involvement of TLR2 or TLR4/CD14 is examined using blocking antibodies to these molecules (eBioscience).

Epithelial cell production of cytokines. The airway epithelial cell line, BEAS-2B, is stimulated with different concentrations of recombinant proteins for 24 hours, similarly to *Alternaria* crude extract experiments in FIGS. 10 and 11. Cytokines, including IL-8 and IL-6, released into supernatant are measured by ELISA. The epithelial cells' PAR-2 is analyzed similarly to the analysis for eosinophils.

Cytokine responses and airway eosinophilia in mouse airways in vivo. Naïve mice are exposed intranasally to recombinant proteins (1 µg-100 µg/challenge) on days 0, 3, and 6 (see FIGS. 16 and 20). At 12 hours after the first challenge, on day 5, or day 8, the trachea is cannulated, and the lung is lavaged with 0.5 mL of HBSS. Total numbers of cells and differentials in BAL fluids are determined. Supernatants are collected, and the concentrations of cytokines (IL-5, IL-4, IL-13, IFN-γ) are measured by ELISA. Tissue samples of the lungs are examined histologically. Blood is collected by cardiac puncture on day 8 to quantitate IgE and IgG antibodies to recombinant proteins.

Cellular and humoral immune responses by CRS patients. PBMC are isolated from normal individuals and CRS patients by using the same criteria as described above. PBMC are incubated with serial dilutions of recombinant proteins for 24 hours (for IL-4), for 96 hours (for IL-5, IL-13, and IFN-γ), or for 168 hours for CFSE-based CD4+ T cell proliferation assay as described above. Serum concentrations of IgE, IgG, and IgG4 antibody to recombinant proteins are measured by immunoassay and western blot.

Development of *A. alternata* Knockout (KO) Mutants for Specific Immunostimulatory Proteins and Analyses of Immune Responses In Vitro and In Vivo with Whole Fungi and Fungal Products.

KO mutants are generated for each candidate immunostimulatory protein. First, the secreted products from KO *A. alternata* are used to deduce whether the absence of a specific protein significantly affects the activation of immune cells in vitro and in vivo. Second, similar experiments with whole fungus (i.e., fungal spores and fungal hyphae) are compare the immune responses triggered by KO to the wild type.

Fungal mutant generation. The LME approach is used as described above to disrupt the target genes. The LME constructs consistently produce stable transformants for diverse categories of genes. Typically, when using the LME constructs, 100% of the transformants are targeted gene disrupt-

28

tion mutants compared to inconsistent transformation and usually less than 10% targeted gene disruption with circular plasmid disruption constructs. All mutants are subjected to molecular characterization to confirm that gene(s) are disrupted.

In vitro and in vivo assays. Wild-type and KO *Alternaria* are cultured in liquid medium. Proteins released from these fungi into supernatants are analyzed for their immunostimulatory activities in vitro with eosinophils and BEAS-2B cells and in vivo mouse airways as described above. Spores are collected from wild-type and KO *Alternaria*. These spores are cultured in vitro in HBSS medium with airway mucin and allowed to germinate. Eosinophils are added, and their responses to wild-type and KO *Alternaria* are examined as in FIG. 13.

Example 10

Inhibiting *Alternaria*-Induced Eosinophilic Degranulation

To monitor eosinophil function in response to extracts from *Alternaria*, degranulation of human eosinophils was measured by quantitating released eosinophil-derived neurotoxin (EDN) and/or MBP. In brief, freshly isolated eosinophils were suspended in HBSS with 25 mM HEPES and 0.01% gelatin at 5×10⁵ cells/mL. Eosinophils and stimuli were incubated in 96-well tissue culture plates for 3 hours at 37°C and 5% CO₂. Cell-free supernatants were stored at -20°C. A specific RIA quantitated eosinophil degranulation by measuring the concentration of EDN in the supernatants. The following inhibited *Alternaria*-induced eosinophilic degranulation: CV6209 (PAF receptor antagonist), heparin, EDTA, EGTA, pepstatin agarose, PAR2-inhibitory peptide, Jasplakinolide (actin inhibitor), and Lanthunum (Ca channel inhibitor). The following did not inhibit eosinophilic degranulation: Chymostatin, Chloroquine, Phosphoramidon, APSF, Calpastatin, Antipain, Bestatin, Leupeptin, Pefabloc SC, Aprotinin, Cytochalasin B, Colchitin, E64, Calpain inhibitor, SB203580 (p38 MAPK inhibitor), Genistein, Wortmannin, Ro-31-8220, Rottlerin, GF109203X, PD98059 (ERK inhibitor), Cyclosporin A, FK 506, W-7, and TLCK.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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Val Lys Ser Asn Asp Trp Tyr Ser Gln Cys Ile Asn Gly Gly Gly Asn
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Ala Pro Ala Pro Pro Ala Ala Thr Gly Val Ala Pro Ala Pro Val Ile
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								70							
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Asn	Ile	Phe	Ser	Thr	Gln	Asp	His	Ala	Ala	Ala	Ile	Ala	Ala	Ala	
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									90						
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Gly	Val	Pro	Val	Phe	Ala	Trp	Lys	Gly	Glu	Thr	Glu	Glu	Tyr	Glu	
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									105						
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										135					
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 aatggtagca agacgcctct gaagcagtct gagctccaac agcccaacct tggctatgaa 960
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 aacaaccaggc acttcgttacc tatcgtggat gcccgcacatc acatcccgaa cccacagaac 1440
 gctagtgacg cttatgatac ctacgctcgc ggaaatgaat ctgatgtatt cctgaggaat 1500

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cctgatggta gtcagttacat tggcgctgtg tggcctggat acaccgtctt cccagactgg 1560
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 ccgtacagcg gtttctgggt cgatatgact gaagtctcct cgttctgcgt cggttccgtc 1680
 ggttccggta atgttacctt gaaccctgtt catccaccc tctccctccc tggcgaggtg 1740
 ggcaacgtca ttttcgacta tccagaaggc ttcaacatca ccaacgcaac tgaggccgt 1800
 tcggcttcag ccggcggttc gagccaggcc gcaccggcg cgcctacggg ggaggctgt 1860
 acgaccacta gctacttccg atcaacgcot acacctggtg tgcgcaacgt caactaccct 1920
 ccatacgtca tcaaccatgt ccaatccgga gctgatctt cgttccacgc agtcagtcct 1980
 aatgcaacac atcagaatgg cggtgaagag tacgtatgtac acaaccttta tggtcaccag 2040
 atcatcaatg ccacctacca gggctttctt caagtcttcc ctggaaagcg cccgtttatc 2100
 atcggacgtt ccacctttgc tggtagcgga aagtggccg gtcactgggg tgggacaac 2160
 gggcttataat gttttttcgg atccctcagg ctctgtcggtt ctgcgttttc 2220
 ggtattccca tggcgccgcgacacttgc ggattcaacg gcaacactaa tatggaaactt 2280
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 aaggacacga acgcgcttgc caacgtgacc attctgggtt ctccttcagt tggacaggtc 3000
 aagttgaatg gcgagacaat cgatgcaagc aagggtgagct acaactctac tagcagcg 3060
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 ctaagctggg agtaa 3135

<210> SEQ_ID NO 8

<211> LENGTH: 1044

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 8

Met	Ala	Pro	Asn	Thr	Gly	Ala	Val	Asp	Ser	Thr	Thr	Val	Arg	Tyr	Lys
1															

5	10	15
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Arg	Thr	Lys	Ser	Gln	Trp	Val	Pro	Glu	Asp	Val	Gln	Ala	Ala	Lys
20														

25	30
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Trp	Phe	Ser	Thr	Thr	Ile	Met	Ser	Arg	Ser	Ser	Phe	Lys	Gln	Val	Ser
35															

40	45
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Thr	Leu	Leu	Ser	Ser	Phe	Leu	Ala	Leu	Thr	Ala	Gly	Gln	Thr	Pro	Val
50															

55	60
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Ser	Ser	Ser	Asp	Gly	Gly	Trp	Ser	Thr	Leu	Ala	Gly	Thr	Pro	Thr
65														

70	75	80
----	----	----

Ala	Phe	Arg	Ser	Val	Phe	Thr	Leu	Pro	Pro	Ser	Val	Asp	Gln	Gly	Val
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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85	90	95	
Glu Gln Ile Pro Asn Ile Tyr Asp Pro Gln Ala Val Asn Ala Gln Asp			
100	105	110	
Val Cys Pro Gly Tyr Arg Ala Ser Gly Leu Glu Gln Gly His Arg Gly			
115	120	125	
Leu Ser Ala Thr Leu Thr Leu Ala Gly Ala Ala Cys Asn Ala Tyr Gly			
130	135	140	
Thr Asp Ile Glu Glu Leu Asp Leu Lys Val Glu Tyr Gln Ser Lys Gly			
145	150	155	160
Arg Leu Ala Val Ser Ile Val Pro Lys His Leu Asp Ala Ser Asn Gln			
165	170	175	
Ser Gln Trp Ile Val Pro Glu Asp Leu Ile Pro Arg Pro Gln Ala Glu			
180	185	190	
Asp Ser Ser Glu Gly Thr Asp Leu Lys Phe Asp Trp Gly Asn Glu Pro			
195	200	205	
Ser Phe Trp Phe Ser Val Gly Arg Arg Ser Thr Gly Asp Val Ile Phe			
210	215	220	
Thr Thr Gln Gly Thr Lys Leu Ile Tyr Glu Asn Gln Phe Val Glu Phe			
225	230	235	240
Val Asn Asn Leu Pro Glu Asp Tyr Asn Leu Tyr Gly Leu Gly Glu Arg			
245	250	255	
Ile His Gly Leu Arg Leu Asn Asn Phe Thr Ala Thr Ile Tyr Ala			
260	265	270	
Ala Asp Val Gly Asp Pro Ile Asp Arg Asn Leu Tyr Gly Ser His Pro			
275	280	285	
Phe Tyr Leu Glu Thr Arg Tyr Phe Glu Lys Gly Ser Asn Gly Ser Lys			
290	295	300	
Thr Pro Leu Lys Gln Ser Glu Leu Gln Gln Pro Asn Leu Gly Tyr Glu			
305	310	315	320
Ser Lys Pro Ala Gly Ser Pro Tyr Glu Ser Arg Ser His Gly Val Tyr			
325	330	335	
Tyr Arg Asn Thr His Gly Met Asp Val Val Met Lys Pro Asp His Leu			
340	345	350	
Thr Trp Arg Thr Leu Gly Gly Ala Ile Asp Leu Phe Phe Tyr Glu Gly			
355	360	365	
Pro Ser Gln Pro Glu Val Thr Lys Glu Tyr Gln Lys Ser Ala Ile Gly			
370	375	380	
Leu Pro Ala Met Gln Gln Tyr Trp Thr Leu Gly Phe His Gln Cys Arg			
385	390	395	400
Trp Gly Tyr Arg Asn Trp Thr Glu Thr Arg Glu Ile Val Glu Thr Met			
405	410	415	
Arg Ala Phe Asn Ile Pro Met Glu Thr Ile Trp Leu Asp Ile Asp Tyr			
420	425	430	
Met Asp Gln Tyr Arg Asp Phe Thr Leu Asp Pro Val Ser Phe Pro Pro			
435	440	445	
Ser Asp Val Lys Asp Phe Phe Asp Trp Leu His Gly Asn Asn Gln His			
450	455	460	
Phe Val Pro Ile Val Asp Ala Ala Ile Tyr Ile Pro Asn Pro Gln Asn			
465	470	475	480
Ala Ser Asp Ala Tyr Asp Thr Tyr Ala Arg Gly Asn Glu Ser Asp Val			
485	490	495	
Phe Leu Arg Asn Pro Asp Gly Ser Gln Tyr Ile Gly Ala Val Trp Pro			
500	505	510	

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Gly Tyr Thr Val Phe Pro Asp Trp Leu Ser Ser Asn Gly Val Ala Trp
515 520 525

Trp Val Lys Glu Met Val Glu Trp Tyr Lys Glu Val Pro Tyr Ser Gly
530 535 540

Phe Trp Val Asp Met Thr Glu Val Ser Ser Phe Cys Val Gly Ser Cys
545 550 555 560

Gly Ser Gly Asn Val Thr Leu Asn Pro Ala His Pro Pro Phe Ser Leu
565 570 575

Pro Gly Glu Val Gly Asn Val Ile Phe Asp Tyr Pro Glu Gly Phe Asn
580 585 590

Ile Thr Asn Ala Thr Glu Ala Ala Ser Ala Ser Ala Gly Ala Ser Ser
595 600 605

Gln Ala Ala Pro Ala Ala Pro Thr Glu Glu Ala Ala Thr Thr Thr Ser
610 615 620

Tyr Phe Arg Ser Thr Pro Thr Pro Gly Val Arg Asn Val Asn Tyr Pro
625 630 635 640

Pro Tyr Val Ile Asn His Val Gln Ser Gly Ala Asp Leu Ala Val His
645 650 655

Ala Val Ser Pro Asn Ala Thr His Gln Asn Gly Val Glu Glu Tyr Asp
660 665 670

Val His Asn Leu Tyr Gly His Gln Ile Ile Asn Ala Thr Tyr Gln Gly
675 680 685

Leu Leu Gln Val Phe Pro Gly Lys Arg Pro Phe Ile Ile Gly Arg Ser
690 695 700

Thr Phe Ala Gly Ser Gly Lys Trp Ala Gly His Trp Gly Gly Asp Asn
705 710 715 720

Ala Ser Lys Trp Ala Tyr Met Phe Phe Ser Ile Pro Gln Ala Leu Ser
725 730 735

Phe Ser Leu Phe Gly Ile Pro Met Phe Gly Ala Asp Thr Cys Gly Phe
740 745 750

Asn Gly Asn Thr Asn Met Glu Leu Cys Ala Arg Trp Met Gln Leu Ser
755 760 765

Ala Phe Phe Pro Phe Tyr Arg Asn His Asn Val Leu Ser Ala Ile Pro
770 775 780

Gln Glu Pro Tyr Arg Trp Asp Ala Val Ala Ser Ala Ser Arg Thr Ala
785 790 795 800

Met His Ile Arg Tyr Ser Leu Leu Pro Tyr Met Tyr Thr Leu Phe Asn
805 810 815

Asp Ala His Thr Thr Gly Ser Thr Val Met Arg Ala Leu Ala Trp Glu
820 825 830

Phe Pro Asn Glu Pro Gln Leu Ala Gly Val Asp Thr Gln Phe Met Leu
835 840 845

Gly Pro Asn Ile Leu Ile Thr Pro Val Leu Glu Pro Gln Val Asp Thr
850 855 860

Val Asn Gly Val Phe Pro Gly Ile Ile Asp Gly Glu Ser Trp Phe Asp
865 870 875 880

Trp Tyr Ser Gly Glu Arg Val Glu Ala Glu Ala Gly Val Asn Thr Thr
885 890 895

Ile Ser Ala Pro Leu Gly His Ile Pro Val Tyr Ile Arg Gly Gly Ser
900 905 910

Val Leu Pro Ile Gln Glu Pro Gly Tyr Thr Thr Glu Ser Arg Lys
915 920 925

Asn Pro Trp Gly Leu Ile Val Ala Leu Ser Ala Asp Gly Thr Ala Ser
930 935 940

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Gly Asn Leu Tyr Val Asp Asp Gly Glu Ser Leu Glu Pro Glu Ser Cys
945 950 955 960

Leu Asp Val Thr Phe Ala Ala Met Asn Gly Gln Leu Lys Ala Asp Val
965 970 975

Glu Gly Lys Phe Lys Asp Thr Asn Ala Leu Ala Asn Val Thr Ile Leu
980 985 990

Gly Ala Pro Ser Val Gly Gln Val Lys Leu Asn Gly Glu Thr Ile Asp
995 1000 1005

Ala Ser Lys Val Ser Tyr Asn Ser Thr Ser Ser Val Leu Lys Leu Ser
1010 1015 1020

Gly Leu Asn Asp Leu Thr Ser Gly Gly Ala Trp Gln Gly Ser Trp Thr
1025 1030 1035 1040

Leu Ser Trp Glu

<210> SEQ ID NO 9

<211> LENGTH: 1869

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 9

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gtatccagtc	ctttccatat	tgaggggcaac	gagggtgtcg	agcatctcca	tacggtagca	120
gagggatgga	gagaggttgg	tgctccagcg	cctgagcata	agctgcattt	ccgcattgca	180
gtgcgcgtcg	ccaaccgcga	tgtatttcaa	aggacgctca	tggaggttgc	gactcctagc	240
caccctcgct	acggtcagca	cctaaagcga	gacgaactga	agcatctcat	caagcctaga	300
gccgactcga	ctgcaagtgt	gcttacctgg	ctcgagcaat	ccggtatcga	agcgcgagac	360
atccagaacg	acggcgagtg	gatcaacttt	ctcgcacccg	tgaagcgcgc	cgagcagatg	420
atgggttacca	cgttcaagac	ctaccagat	caagcgcgtc	cagcgtctaa	gagaactcgc	480
tcgttgggt	actctgtgcc	cttggacgtc	cgcagtcata	ttgatatgtat	ccagcctacc	540
actcgcttcg	gtgaaatccg	cccccgatcc	agccaagtcc	ttacgcaaaa	gaccgctccc	600
ttctcggtgc	ttgctgtcaa	tgccacgtgc	aacacaagga	tcacgccccga	ttgtctcgca	660
gatctgtaca	acttcaagga	ttacaacgtt	agtgacaaag	ccatgtgtac	aatcgggttg	720
agcgggttcc	tcgagcagta	cgcgggttcc	aacgatctcg	accagttcat	ccaaagattt	780
gtcccccggcc	ttgcgggtaa	aacgttcaaa	gtccagttata	tcaatggtaa	gatcgtgtca	840
ttgttacctc	gttatctca	gctaacgttc	gtagacgggc	cgttccctca	aaactcaacg	900
gccaacacgc	ttgaggctaa	cctcgacatc	cagtatacac	ctgggtctgg	gtcgtctaaag	960
atttcaacca	ctttctacac	tgttccagga	cgaggactgt	tggtccccga	ccttgaccaa	1020
cctgtatctcg	aggacgagga	gctgcctgaa	gtactgacga	cgtcgtacgg	tgagacggag	1080
cagagegttc	ctgcggagta	tgccaagaag	gtttgtgaca	tgatcgccca	gctcggtact	1140
cgtgggtct	cggtcatctt	cgaggatgaa	tccaccacag	ccagcgggtga	tactggtcca	1200
ggctctgcct	gtcagagcaa	tgacggcaag	aacgctaccc	gtctcaacc	aatcttccca	1260
gcttcatgcc	cctacgttac	ttcagtcgtt	ggcacgtttt	gagtggaaacc	cgaacgtgt	1320
gttgagttct	cttctgggtgg	cttctctgtat	ctctggtctc	gcccgccgta	ccaagagaag	1380
gcagtgtactg	actaccttgg	caaactgggc	tcgcaatggc	aaggtttga	caacgcac	1440
ggacgaggtt	ttccagatgt	cgcggctcaa	ggaaaggat	ttcaggatcat	tgataagctt	1500
ggcttgcgt	ctgttggagg	aaccagcgcc	tcagcgcctc	tcttcgttgc	ggtcattgcg	1560

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cttctgaaca acgctcgaaa ggcggctggatgcgccttcgc tggcgtttt gaacccttgg 1620
 atctacgagc aaggctacaa gggcatgaat gatattgtcg agggaggctc gcgcggatgc 1680
 actggtcgtctatccatcgactcg tgccttacgc ctcctggaaat 1740
 gcgaccgagg gctgggatcc cgtaaccggatcac gcaacttga gcagatgttt 1800
 cgcccttcgaat acgcccga atacggtgcg cgtcgcttc ggcgtggtag cctccgtggaa 1860
 gagggcttag 1869

<210> SEQ_ID NO 10
 <211> LENGTH: 622
 <212> TYPE: PRT
 <213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 10

Met Arg Tyr Thr Ala Thr Phe Thr Gly Val Leu Ala Ile Ala Gly Val
 1 5 10 15

Ser Ala Trp Ser Val Ser Ser Pro Phe His Ile Glu Gly Asn Glu Val
 20 25 30

Val Glu His Leu His Thr Val Pro Glu Gly Trp Arg Glu Val Gly Ala
 35 40 45

Pro Ala Pro Glu His Lys Leu His Phe Arg Ile Ala Val Arg Ser Ala
 50 55 60

Asn Arg Asp Val Phe Glu Arg Thr Leu Met Glu Val Ser Thr Pro Ser
 65 70 75 80

His Pro Arg Tyr Gly Gln His Leu Lys Arg Asp Glu Leu Lys His Leu
 85 90 95

Ile Lys Pro Arg Ala Asp Ser Thr Ala Ser Val Leu Thr Trp Leu Glu
 100 105 110

Gln Ser Gly Ile Glu Ala Arg Asp Ile Gln Asn Asp Gly Glu Trp Ile
 115 120 125

Asn Phe Leu Ala Pro Val Lys Arg Ala Glu Gln Met Met Gly Thr Thr
 130 135 140

Phe Lys Thr Tyr Gln Ser Gln Ala Arg Pro Ala Leu Lys Arg Thr Arg
 145 150 155 160

Ser Leu Gly Tyr Ser Val Pro Leu Asp Val Arg Ser His Ile Asp Met
 165 170 175

Ile Gln Pro Thr Thr Arg Phe Gly Glu Ile Arg Pro Glu Phe Ser Gln
 180 185 190

Val Leu Thr Gln Lys Thr Ala Pro Phe Ser Val Leu Ala Val Asn Ala
 195 200 205

Thr Cys Asn Thr Arg Ile Thr Pro Asp Cys Leu Ala Asp Leu Tyr Asn
 210 215 220

Phe Lys Asp Tyr Asn Val Ser Asp Lys Ala Asp Val Thr Ile Gly Val
 225 230 235 240

Ser Gly Phe Leu Glu Gln Tyr Ala Arg Phe Asn Asp Leu Asp Gln Phe
 245 250 255

Ile Gln Arg Phe Ala Pro Ser Leu Ala Gly Lys Thr Phe Lys Val Gln
 260 265 270

Ser Ile Asn Gly Lys Met Gln Ser Leu Leu Pro Arg Tyr Leu Gln Leu
 275 280 285

Thr Phe Val Asp Gly Pro Phe Pro Gln Asn Ser Thr Ala Asn Ser Val
 290 295 300

Glu Ala Asn Leu Asp Ile Gln Tyr Thr Ala Gly Leu Val Ser Pro Lys
 305 310 315 320

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Ile Ser Thr Thr Phe Tyr Thr Val Pro Gly Arg Gly Leu Leu Val Pro
325 330 335

Asp Leu Asp Gln Pro Asp Leu Glu Asp Glu Glu Leu Pro Glu Val Leu
340 345 350

Thr Thr Ser Tyr Gly Glu Thr Glu Gln Ser Val Pro Ala Glu Tyr Ala
355 360 365

Lys Lys Val Cys Asp Met Ile Gly Gln Leu Gly Thr Arg Gly Val Ser
370 375 380

Val Ile Phe Glu Asp Glu Ser Thr Thr Ala Ser Gly Asp Thr Gly Pro
385 390 395 400

Gly Ser Ala Cys Gln Ser Asn Asp Gly Lys Asn Ala Thr Arg Leu Gln
405 410 415

Pro Ile Phe Pro Ala Ser Cys Pro Tyr Val Thr Ser Val Gly Gly Thr
420 425 430

Phe Gly Val Glu Pro Glu Arg Ala Val Glu Phe Ser Ser Gly Gly Phe
435 440 445

Ser Asp Leu Trp Ser Arg Pro Ala Tyr Gln Glu Lys Ala Val Thr Asp
450 455 460

Tyr Leu Gly Lys Leu Gly Ser Gln Trp Gln Gly Leu Tyr Asn Ala Asn
465 470 475 480

Gly Arg Gly Phe Pro Asp Val Ala Ala Gln Gly Lys Gly Phe Gln Val
485 490 495

Ile Asp Lys Leu Gly Leu Ser Ser Val Gly Gly Thr Ser Ala Ser Ala
500 505 510

Pro Val Phe Ala Ser Val Ile Ala Leu Leu Asn Asn Ala Arg Leu Ala
515 520 525

Ala Gly Met Pro Ser Leu Gly Phe Leu Asn Pro Trp Ile Tyr Glu Gln
530 535 540

Gly Tyr Lys Gly Met Asn Asp Ile Val Glu Gly Gly Ser Arg Gly Cys
545 550 555 560

Thr Gly Arg Ser Ile Tyr Ser Gly Leu Pro Thr Arg Leu Val Pro Tyr
565 570 575

Ala Ser Trp Asn Ala Thr Glu Gly Trp Asp Pro Val Thr Gly Tyr Gly
580 585 590

Thr Pro Asp Phe Glu Gln Met Leu Arg Leu Ser Thr Thr Pro Gln Tyr
595 600 605

Gly Ala Arg Arg Val Arg Arg Gly Ser Leu Arg Gly Glu Ala
610 615 620

<210> SEQ_ID NO 11

<211> LENGTH: 1782

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 11

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ccatttcgaaa agctttcga tggcccagag ggatgaaacg tccaaaggccc tgcacggct 120
gcgcacacgc tcaagctcca ggtcgcgctc cagcaaggcg ataccgcccc ctttgagcag 180
accgtcatgg aaatgtccac cccctccaat gcaaagtacg ggcagcacct tgagtccac 240
gagccaaatga agcgcatgct catgcccagt gaggagaccg tttcctccgt ctcttcctgg 300
ctcaaggctg ccggtatcaa gaacttttag attgacgccc attgggtgac cttcaagaca 360
accgttggtg ttgccaacga gcttcctcaga accaagttct cctgggttgt cagcgaggag 420

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agtacgcctc gcaaagtctt ccgcacgctc gagtactctg tgcccgacga cattggccac	480
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gagcgcgaga tcttcggtat tgcgctagcc tcttccccca acgtactgt caactgtgtat	600
gcgtccatca ctccccagtg cttgaaggcag ctctacaaga ttgactacac tcccggcccc	660
aagagtgccg gtaaggcagc ttgcgttcc tatctcgagg agtacgcgcg ctacagcgac	720
ctcgccctct tcgaggagaa cgtccccc gaggctgtgg gccagaactt ctccgttgtt	780
caattcaacg gcggcgttggaa cgaccaagcc tctgcgcacg acagtggcga ggccaaacttg	840
gatttgcagt acatgctcgg tcttgccag cccctgcctg ttatttagtta tagcactgg	900
ggacgtggcc catggatcgc tgacccgcac cagcctgcac aggctgacag cgccaaacgag	960
ccctaccccg agttcccttca gtcgggtgtc aagctccac agagcgatct tccccaggtc	1020
atctccacgt cttacggcga gaacgaaaca akgatccca agtcttacgc tctcagcgtc	1080
tgcacccctct tcgctcaact tggtagccgt ggtgtctctg tcatcttctc atctgggtat	1140
tccggtaccg gatccgcctg ccttccaac gacggcaaga acactacaa gttccagcc	1200
cagtagccctcg ctgcgtgcctt attcgtcacc tccgtcggtt caactcgcta cctcaacgag	1260
actgcgcactt tcttcctc tgggtgttcc tccgactact ggaagcgccc cagctaccag	1320
gatgtatgcgcg tcaaggcata cttgcataa ctggccaga agaacaagcc ctacttcaac	1380
cggccacgggc gcggttcc ggacgtctcg gcccagggtt cccgttacag ggtctacgac	1440
aagggttctc tcaaggggta ccagggtact tcatgtctcg ctcccgcttt cggcggtatc	1500
gtcgctctcc tcaatgacgc gctgtgttcc gccaagaagg ctgcttgg tttctgtac	1560
ccctgtcttt actccaaaccc ggatgcgcctc aacgatatcg ttcttgggtt cggcggat	1620
tgtgtatggcc acgcgcgcctt caatggcaag ccgaacggta gcccgttat cccgtacgcg	1680
agctggaaaccc ccactgcggg atggaccca gttccggat tgggcacgcc aaactcccc	1740
aaqtgtctca aqgctqctt tccqctcaaq tacaqqctt aq	1782

<210> SEQ ID NO 12

<211> LENGTH: 593

<212> TYPE: PRT

<213> ORGANISM: *Alternaria brassicicola*

<400> SEQUENCE: 12

Met Ala Pro Val Leu Ser Phe Ile Val Gly Ser Leu Leu Ala Leu Gln
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Ala Phe Ala Glu Pro Phe Glu Lys Leu Phe Asp Val Pro Glu Gly Trp
20 25 30

Lys Leu Gln Gly Pro Ala Ser Ala Ala His Thr Leu Lys Leu Gln Val
35 40 45

Ala Leu Gln Gln Gly Asp Thr Ala Gly Phe Glu Gln Thr Val Met Glu
50 55 60

Met	Ser	Thr	Pro	Ser	Asn	Ala	Lys	Tyr	Gly	Gln	His	Phe	Glu	Ser	His
65					70					75					80

Glu Gln Met Lys Arg Met Leu Met Pro Ser Glu Glu Thr Val Ser Ser
 85 90 95

Val Ser Ser Trp Leu Lys Ala Ala Gly Ile Lys Asn Phe Glu Ile Asp
 100 105 110

Ala Asp Trp Val Thr Phe Lys Thr Thr Val Gly Val Ala Asn Glu Leu
 115 120 125

Leu Arg Thr Lys Phe Ser Trp Phe Val Ser Glu Glu Ser Thr Pro Arg
130 135 140

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Lys Val Leu Arg Thr Leu Glu Tyr Ser Val Pro Asp Asp Ile Ala Asp
145 150 155 160

His Ile Asn Leu Val Gln Pro Thr Thr Arg Phe Ala Ala Ile Arg Ala
165 170 175

Asn His Glu Thr Glu Arg Glu Ile Phe Gly Ile Ala Leu Ala Ser Ser
180 185 190

Pro Asn Val Thr Val Asn Cys Asp Ala Ser Ile Thr Pro Gln Cys Leu
195 200 205

Lys Gln Leu Tyr Lys Ile Asp Tyr Thr Pro Asp Pro Lys Ser Gly Ser
210 215 220

Lys Ala Ala Phe Ala Ser Tyr Leu Glu Glu Tyr Ala Arg Tyr Ser Asp
225 230 235 240

Leu Ala Leu Phe Glu Glu Asn Val Leu Pro Glu Ala Val Gly Gln Asn
245 250 255

Phe Ser Val Val Gln Phe Asn Gly Gly Leu Asn Asp Gln Ala Ser Ala
260 265 270

Asp Asp Ser Gly Glu Ala Asn Leu Asp Leu Gln Tyr Met Leu Gly Leu
275 280 285

Ala Gln Pro Leu Pro Val Ile Glu Tyr Ser Thr Gly Gly Arg Gly Pro
290 295 300

Trp Ile Ala Asp Leu Asp Gln Pro Asp Glu Ala Asp Ser Ala Asn Glu
305 310 315 320

Pro Tyr Leu Glu Phe Leu Gln Ser Val Leu Lys Leu Pro Gln Ser Asp
325 330 335

Leu Pro Gln Val Ile Ser Thr Ser Tyr Gly Glu Asn Glu Gln Ser Val
340 345 350

Pro Lys Ser Tyr Ala Leu Ser Val Cys Asn Leu Phe Ala Gln Leu Gly
355 360 365

Ser Arg Gly Val Ser Val Ile Phe Ser Ser Gly Asp Ser Gly Thr Gly
370 375 380

Ser Ala Cys Leu Ser Asn Asp Gly Lys Asn Thr Thr Lys Phe Gln Pro
385 390 395 400

Gln Tyr Pro Ala Ala Cys Pro Phe Val Thr Ser Val Gly Ser Thr Arg
405 410 415

Tyr Leu Asn Glu Thr Ala Thr Phe Phe Ser Ser Gly Gly Phe Ser Asp
420 425 430

Tyr Trp Lys Arg Pro Ser Tyr Gln Asp Asp Ala Val Lys Ala Tyr Leu
435 440 445

His Gln Leu Gly Gln Lys Asn Lys Pro Tyr Phe Asn Arg His Gly Arg
450 455 460

Gly Phe Pro Asp Val Ser Ala Gln Gly Ser Gly Tyr Arg Val Tyr Asp
465 470 475 480

Lys Gly Ser Leu Lys Gly Tyr Gln Gly Thr Ser Cys Ser Ala Pro Ala
485 490 495

Phe Gly Gly Ile Val Ala Leu Leu Asn Asp Ala Arg Leu Arg Ala Lys
500 505 510

Lys Pro Ala Leu Gly Phe Leu Asn Pro Leu Leu Tyr Ser Asn Pro Asp
515 520 525

Ala Leu Asn Asp Ile Val Leu Gly Gly Ser Thr Gly Cys Asp Gly His
530 535 540

Ala Arg Phe Asn Gly Lys Pro Asn Gly Ser Pro Val Ile Pro Tyr Ala
545 550 555 560

Ser Trp Asn Ala Thr Ala Gly Trp Asp Pro Val Ser Gly Leu Gly Thr

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565

570

575

Pro Asn Phe Pro Lys Leu Leu Lys Ala Ala Leu Pro Ala Arg Tyr Lys
 580 585 590

Ala

<210> SEQ ID NO 13
 <211> LENGTH: 1112
 <212> TYPE: DNA
 <213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 13

atgtttgccaaactactctcatgagcgcgctgctcagcgctgcactgccgaggcatct	60
gggacggtcgcttcaacgacatgacctctctaccgaactctccgactggtccttcca	120
accccgctcgccagctaccatactacatccacggctcgttccgtactacgtaa	180
acctcgccgcaccttcaagaaccccgccgacacagcttccaagcaaggtgtcaagatca	240
ccatcgacgagaactgcaaaaatggacggccaaaccatgtctgcacccag	300
agaccaaggccgcatcaaacaaaggcaaacttactaccaattctccgtcaagacaacgg	360
ctgagaacgcgcccggccaccacgaaaccatgtcgatgttcttcgagagccacttca	420
ccgagttgaaatggcgcttctgggttcttcgacacaccaatggcacgttggt	480
gcgtctccaaatggacgttagctcgatgttgcgttgcacacgttgcctacgaaa	540
tgcactttgatggcggttccgtcgatttgcactccacccgtgtatgtcaagc	600
agacagctggcccggtcgatgttgcacacttcttcgactggcatcttgcgt	660
tgcgtgggtgcgggttaacggcgacaaggatggtgcgttgcgttgcgttgcgt	720
ttggtagtggagctgtggatggccccagaaaaggctgttgccagtgtgtgcacctt	780
ccaatgtcgtttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt	840
tctcctccatcgctgggtgtcgagacttgcgtatctccactgtgttgcgttgcgt	900
ccactcgacttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt	960
ctcccgatcgactcccgccgcctttctatctctgtgttgcgttgcgttgcgttgcgt	1020
ctgctgtggccggctctgacgcacgttcccgaggatgttgcgttgcgttgcgttgcgt	1080
cttggctcaaggctaaactggcaagaactaa	1112

<210> SEQ ID NO 14
 <211> LENGTH: 370
 <212> TYPE: PRT
 <213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 14

Met Phe Ala Lys Thr Thr Leu Met Ser Ala Leu Leu Ser Ala Ala Ser	
1 5 10 15	
Ala Glu Val Ile Trp Asp Gly Arg Phe Asn Asp Met Thr Ser Ser Thr	
20 25 30	
Glu Leu Ser Asp Trp Ser Phe Ser Asn Pro Val Gly Ser Tyr Gln Tyr	
35 40 45	
Tyr Ile His Gly Pro Gly Ser Val Thr Asp Tyr Val Asn Leu Gly Ala	
50 55 60	
Thr Phe Lys Asn Pro Ala Asp Thr Ala Ser Lys Gln Gly Val Lys Ile	
65 70 75 80	
Thr Ile Asp Glu Thr Ala Lys Trp Asn Gly Gln Thr Met Leu Arg Thr	
85 90 95	
Glu Leu Ile Pro Glu Thr Lys Ala Ala Ile Asn Lys Gly Lys Val Tyr	

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110

Tyr His Phe Ser Val Lys Thr Thr Ala Glu Asn Ala Pro Thr Ala Thr
 115 120 125

Asn Glu His Gln Val Ala Phe Phe Glu Ser His Phe Thr Glu Leu Lys
 130 135 140

Tyr Gly Ala Ser Gly Ser Ser Asn Thr Asn Leu Gln Trp His Val Gly
 145 150 155 160

Gly Val Ser Lys Trp Asp Val Glu Leu Val Ala Asp Glu Trp His Asn
 165 170 175

Val Ala Tyr Glu Ile Asp Phe Asp Ala Gly Ser Val Ala Phe Trp His
 180 185 190

Ser Thr Gly Ala Asp Glu Leu Lys Gln Thr Ala Gly Pro Phe Asp Ala
 195 200 205

Ser Thr Ser Ser Asn Gly Ala Asp Trp His Leu Gly Val Leu Arg Leu
 210 215 220

Pro Gly Asn Ala Asp Lys Asp Gly Ala Glu Asp Trp Phe Phe Ser Gly
 225 230 235 240

Val Gly Ser Gly Ala Ala Gly Ala Ala Pro Glu Lys Pro Val Ala Ser
 245 250 255

Ala Ala Ala Pro Ser Asn Val Val Ser Ser Ala Ala Pro Ala Ala Thr
 260 265 270

Thr Ser Lys Ala Ala Val Ala Pro Val Ser Ser Ala Ala Ala Val
 275 280 285

Glu Thr Ser Val Val Ser Ser Thr Ala Ala Ala Ser Ser Thr Ala Val
 290 295 300

Pro Ala Glu Thr Pro Ala Val Ser Ser Ala Ala Ala Ile Ser Ser Ala
 305 310 315 320

Ala Pro Val Glu Thr Pro Ala Ala Ser Ser Thr Ser Ala Val Thr Pro
 325 330 335

Val Ala Thr Pro Thr Ala Val Ala Gly Ser Asp Ala Lys Leu Pro Glu
 340 345 350

Glu Phe Thr Ile Ser Gln Phe Val Ala Trp Leu Lys Ala Lys Thr Gly
 355 360 365

Lys Asn
 370

<210> SEQ ID NO 15

<211> LENGTH: 336

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 15

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atgtctacct ccgagctcgc cacctttac gccgctctca tcctcgctga tgacgggtgc   60
gacatcaactg ccgacaagct ccagtctctc atcaaggccg caaatcgaa ggagggtcgag   120
ccccatctgga cgaccctgtt cgccaaggct cttgagggca aggtatgtcaa ggacctgcta   180
ctgaacgtcg gtcaggccgg cggegctgcc cctgctgccg gaggcgctgc ccctgctgct   240
ggcggtgctg ctgaggccgc accagctgcc gaggagaaga aggaggagga gaaggaggag   300
tcagacgagg acatgggctt cggtctcttc gactaa                           336

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<210> SEQ ID NO 16

<211> LENGTH: 111

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 16

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Met Ser Thr Ser Glu Leu Ala Thr Ser Tyr Ala Ala Leu Ile Leu Ala
 1 5 10 15

Asp Asp Gly Val Asp Ile Thr Ala Asp Lys Leu Gln Ser Leu Ile Lys
 20 25 30

Ala Ala Lys Ile Glu Glu Val Glu Pro Ile Trp Thr Thr Leu Phe Ala
 35 40 45

Lys Ala Leu Glu Gly Lys Asp Val Lys Asp Leu Leu Leu Asn Val Gly
 50 55 60

Ser Gly Gly Ala Ala Pro Ala Ala Gly Gly Ala Ala Pro Ala Ala
 65 70 75 80

Gly Gly Ala Ala Glu Ala Ala Pro Ala Ala Glu Glu Lys Lys Glu Glu
 85 90 95

Glu Lys Glu Glu Ser Asp Glu Asp Met Gly Phe Gly Leu Phe Asp
 100 105 110

<210> SEQ ID NO 17

<211> LENGTH: 654

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 17

atggctgcac ctcagtagac cctgcctccg ctgccatatg catacaatgc attggaggcg 60
 cacatctcag cacagatcat ggagctgcac cacagcaagc accaccagac gtatatcacc 120
 aacctgaatg gtcttctcaa gactcaagcc gaagccgttt ctacctccga catcaatca 180
 caggtttcga tacagcaagg catcaagtcc aacgctggcg gccacatcaa ccactcttc 240
 ttctggcaaa acctcgctcc tgccagctcg ggtgaggctc agagctccgc tgctctgag 300
 ctactcaaac agatcaaggc gacttgggga gacgaggata agttcaaggc agcctcaac 360
 acagcttgc taggcatcca aggaagtgg tggggatgg tggtaagac cgatataggc 420
 aaggaggaga gattgtctat cgtgacgacc aaggaccagg atcctgttgt tggtaaaggc 480
 gaagttccga tcttcggtgt tgacatgtgg gagcatgcgt actatctcca gtaccagaat 540
 ggtaaggctg cttacgtcaa gaatatctgg aatgtcatta actggaagac ggccggaggag 600
 cgttatctgg gatcgccgc agatgcttc agtgtgctga gggcatccat ctaa 654

<210> SEQ ID NO 18

<211> LENGTH: 217

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 18

Met Ala Ala Pro Gln Tyr Thr Leu Pro Pro Leu Pro Tyr Ala Tyr Asn
 1 5 10 15

Ala Leu Glu Pro His Ile Ser Ala Gln Ile Met Glu Leu His His Ser
 20 25 30

Lys His His Gln Thr Tyr Ile Thr Asn Leu Asn Gly Leu Leu Lys Thr
 35 40 45

Gln Ala Glu Ala Val Ser Thr Ser Asp Ile Thr Ser Gln Val Ser Ile
 50 55 60

Gln Gln Gly Ile Lys Phe Asn Ala Gly Gly His Ile Asn His Ser Leu
 65 70 75 80

Phe Trp Gln Asn Leu Ala Pro Ala Ser Ser Gly Glu Ala Gln Ser Ser
 85 90 95

Ala Ala Pro Glu Leu Leu Lys Gln Ile Lys Ala Thr Trp Gly Asp Glu
 100 105 110

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Asp Lys Phe Lys Glu Ala Phe Asn Thr Ala Leu Leu Gly Ile Gln Gly
115 120 125

Ser Gly Trp Gly Trp Leu Val Lys Thr Asp Ile Gly Lys Glu Gln Arg
130 135 140

Leu Ser Ile Val Thr Thr Lys Asp Gln Asp Pro Val Val Gly Lys Gly
145 150 155 160

Glu Val Pro Ile Phe Gly Val Asp Met Trp Glu His Ala Tyr Tyr Leu
165 170 175

Gln Tyr Gln Asn Gly Lys Ala Ala Tyr Val Lys Asn Ile Trp Asn Val
180 185 190

Ile Asn Trp Lys Thr Ala Glu Glu Arg Tyr Leu Gly Ser Arg Ala Asp
195 200 205

Ala Phe Ser Val Leu Arg Ala Ser Ile
210 215

<210> SEQ ID NO 19

<211> LENGTH: 771

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 19

atggggcgtga	tgagtgaaaa	ggttgccgc	tgtatcgacg	agattgagga	atccactctc	60
agcacccgagg	gcaagggtcca	agcccagact	gttattacgg	aagagcttaa	aaagctgctc	120
aagcactgtg	cgaatgcaac	agattgcgtc	tatacggctc	tgcacttgct	tcgtaactcg	180
ctgcataatca	atgagtctaa	tcagggccct	gacatgagca	tcattaaaga	gctgatcgcg	240
gagaacgcgg	tccgggttag	cacgcacgc	aagagctgg	tatggggtgt	cgaaaagtc	300
gtgcttggag	cagtaaccgag	tgcaactatac	gctatcgccg	cgccgtactct	ttatggtacc	360
aacgattttg	gtttggcacc	gcagactaac	accaacagca	tgcaccccca	ggtcatttcc	420
ctcgcccagc	gcccacaagc	ggtgaccaac	ctcacaggcg	aatccactc	catcaaactt	480
gagcatctag	accgcgccta	ccaggagctc	gaaggcgcct	ctgaatctca	cggctccga	540
atcgacaacc	tggtcgaagc	actgggtct	ccaatgcag	acggcaccta	ctattcatct	600
atgcccggaaac	ctgactgcca	accccttagc	gatatcccga	tgatctacgc	aaaccccgat	660
cgccagattg	aacgactgcg	cagcgagctg	cagaccatgc	gtaagaatat	tcatcgcatg	720
gacattcgcc	tcatgaagcg	tctcaataag	atcgaccaac	gtggctgtg	a	771

<210> SEQ ID NO 20

<211> LENGTH: 256

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 20

Met Gly Val Met Ser Glu Lys Val Ala Ser Cys Ile Asp Glu Ile Glu	1	5	10	15		
---	---	---	----	----	--	--

Glu Ser Thr Leu Ser Thr Glu Gly Lys Val Gln Ala Gln Thr Val Ile	20	25	30			
---	----	----	----	--	--	--

Thr Glu Glu Leu Lys Lys Leu Leu Lys His Cys Ala Asn Ala Thr Asp	35	40	45			
---	----	----	----	--	--	--

Cys Val Tyr Thr Ala Leu Asp Leu Leu Arg Asn Ser Leu His Ile Asn	50	55	60			
---	----	----	----	--	--	--

Glu Ser Asn Gln Gly Pro Asp Met Ser Ile Ile Lys Glu Leu Ile Ala	65	70	75	80		
---	----	----	----	----	--	--

Glu Asn Ala Val Arg Leu Ser Thr Pro Arg Lys Ser Trp Leu Trp Gly

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Val Ala Lys Val Val Leu Gly Ala Val Thr Ser Ala Thr Ile Ala Ile
 100 105 110

Ala Ala Ala Tyr Leu Tyr Gly Thr Asn Asp Phe Gly Leu Ala Pro Gln
 115 120 125

Thr Asn Thr Asn Ser Met His Pro Gln Val Ile Ser Leu Val Gln Arg
 130 135 140

Ala Gln Ala Val Thr Asn Leu Thr Gly Glu Ile His Ser Ile Lys Leu
 145 150 155 160

Glu His Leu Asp Arg Arg Tyr Gln Glu Leu Glu Gly Ala Ser Glu Ser
 165 170 175

His Gly Leu Arg Ile Asp Asn Leu Val Glu Ala Leu Gly Ala Pro Asn
 180 185 190

Ala Asp Gly Thr Tyr Tyr Ser Ser Met Pro Lys Pro Asp Cys Gln Pro
 195 200 205

Pro Ser Asp Ile Pro Met Ile Tyr Ala Asn Pro Asp Arg Gln Ile Glu
 210 215 220

Arg Leu Arg Ser Glu Leu Gln Thr Met Arg Lys Asn Ile His Arg Met
 225 230 235 240

Asp Ile Arg Leu Met Lys Arg Leu Asn Lys Ile Asp Gln Arg Gly Leu
 245 250 255

<210> SEQ ID NO 21

<211> LENGTH: 1280

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 21

atgacaacct	tcctcctccg	cgatatccgc	atctttaccg	gcgaggggac	catcgacaaa	60
gggttatattc	acgttcaaaa	tggcaagata	aaggctatcg	gccagataag	cgaggctccg	120
ctggactcag	taaagacata	ctctaaacca	ggtcatacga	ttcttcagg	gttgattgac	180
tgtcacatcc	atgcccacag	ggccgatect	gaagctctac	cccaagccct	gcgccttgg	240
gtgactaccg	tttgcgagat	gcacaacgag	ctggagaacg	tacaaaagct	gaagaacgag	300
accatggcgc	ccgataactgc	ttcatacataag	acagcaggc	aggccgctac	tattgagaat	360
gggtggccta	tacccgtcat	cacggccccac	gacaagactc	cagagactgc	agcggcgatt	420
gcgaaatggc	caaaaactgac	ggatcgggat	agcgtggtg	agttcctgga	atggactgg	480
agagagatgc	accaaaatta	catcaaactc	atgcacgaa	gcggaaactat	catggacgc	540
aatttttagct	atccttcgtt	cgaactgcaa	agtacgtac	ttgcagaagc	caaaaaacgg	600
ggatacttga	ccgtcgcgca	cgctctaagt	atgcgtgaca	cgctcgaggt	tctgaatgca	660
ggtgtcgacg	gccttacgca	tacgttttc	gaccagccgc	caacccagga	actagtagat	720
gcgtacaaaa	agaacaacgc	atgggtcaac	ccgacacttg	ttgcgtatgg	cagcctgacg	780
accgaggggaa	aagagctgca	gcatcaattt	gcacacgatc	ccagggtgaa	agggttgatc	840
aaggaagatc	gtgttaggcaa	catgtgcaag	tgcgtggct	ttgcgtatgg	gggagggaaa	900
gtagaatacg	cataatcaagg	cgtgaaatgg	ctgagagaag	cgggcatcga	catcctgtgt	960
gggagcgact	ccgcgggtcc	ggcagtaggg	acggcatttg	gtctatcgat	gcatcagcga	1020
ttgttatctcc	tcgttaataaa	ggtggaaatg	acacctatag	aggctttacg	ctcagccaca	1080
agectgacgg	cgaagcgctt	ccaatttagg	gatcgtggtc	gtctggcgga	agggctcaac	1140
gcccatttgt	tactggtaga	aggaaatccg	cttgaagaca	ttgatgcgac	gctaaatatc	1200

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cgccggcggtt ggcgggatgg caaccttgtt agcacgttgt tgaaaagctt ggagctggtg 1260
 ttgagcctct attgagttga 1280

<210> SEQ ID NO 22
 <211> LENGTH: 426
 <212> TYPE: PRT
 <213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 22

Met	Thr	Phe	Leu	Leu	Arg	Asp	Ile	Arg	Ile	Phe	Thr	Gly	Glu	Gly
1							5		10			15		

Thr	Ile	Asp	Lys	Gly	Tyr	Ile	His	Val	Gln	Asn	Gly	Lys	Ile	Lys	Ala
							20		25			30			

Ile	Gly	Gln	Ile	Ser	Glu	Ala	Pro	Leu	Asp	Ser	Val	Lys	Thr	Tyr	Ser
							35		40			45			

Lys	Pro	Gly	His	Thr	Ile	Leu	Pro	Gly	Leu	Ile	Asp	Cys	His	Ile	His
							50		55			60			

Ala	Asp	Arg	Ala	Asp	Pro	Glu	Ala	Leu	Pro	Gln	Ala	Leu	Arg	Phe	Gly
							65		70			80			

Val	Thr	Thr	Val	Cys	Glu	Met	His	Asn	Glu	Leu	Glu	Asn	Val	Gln	Lys
							85		90			95			

Leu	Lys	Lys	Gln	Thr	Met	Glu	Pro	Asp	Thr	Ala	Ser	Tyr	Lys	Thr	Ala
							100		105			110			

Gly	Gln	Ala	Ala	Thr	Ile	Glu	Asn	Gly	Trp	Pro	Ile	Pro	Val	Ile	Thr
							115		120			125			

Ala	His	Asp	Lys	Thr	Pro	Glu	Thr	Ala	Ala	Ala	Ile	Ala	Lys	Trp	Pro
							130		135			140			

Lys	Leu	Thr	Asp	Arg	Asp	Ser	Val	Val	Glu	Phe	Leu	Glu	Trp	Thr	Gly
							145		150			155			160

Arg	Glu	Met	Gln	Pro	Asn	Tyr	Ile	Lys	Leu	Met	His	Glu	Ser	Gly	Thr
							165		170			175			

Ile	Met	Gly	Arg	Asn	Phe	Ser	Tyr	Pro	Ser	Phe	Glu	Leu	Gln	Ser	Thr
							180		185			190			

Ile	Ile	Ala	Glu	Ala	Lys	Lys	Arg	Gly	Tyr	Leu	Thr	Val	Ala	His	Ala
							195		200			205			

Leu	Ser	Met	Arg	Asp	Thr	Leu	Glu	Val	Leu	Asn	Ala	Gly	Val	Asp	Gly
							210		215			220			

Leu	Thr	His	Thr	Phe	Phe	Asp	Gln	Pro	Pro	Thr	Gln	Glu	Leu	Val	Asp
							225		230			235			240

Ala	Tyr	Lys	Asn	Asn	Ala	Trp	Val	Asn	Pro	Thr	Leu	Val	Ala	Ile	
							245		250			255			

Gly	Ser	Leu	Thr	Thr	Glu	Gly	Lys	Glu	Leu	Gln	His	Gln	Phe	Ala	His
							260		265			270			

Asp	Pro	Arg	Val	Lys	Gly	Leu	Ile	Lys	Glu	Asp	Arg	Val	Gly	Asn	Met
							275		280			285			

Cys	Lys	Cys	Met	Gly	Phe	Ala	Ala	Glu	Gly	Gly	Lys	Val	Glu	Tyr	Ala
							290		295			300			

Tyr	Gln	Gly	Val	Lys	Gly	Leu	Arg	Glu	Ala	Gly	Ile	Asp	Ile	Leu	Cys
							305		310			315			320

Gly	Ser	Asp	Ser	Ala	Gly	Pro	Ala	Val	Gly	Thr	Ala	Phe	Gly	Leu	Ser
							325		330			335			

Met	His	His	Glu	Leu	Tyr	Leu	Leu	Val	Asn	Lys	Val	Gly	Met	Thr	Pro
							340		345			350			

Ile	Glu	Ala	Leu	Arg	Ser	Ala	Thr	Ser	Leu	Thr	Ala	Lys	Arg	Phe	Gln
							355		360			365			

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Phe Arg Asp Arg Gly Arg Leu Ala Glu Gly Leu Asn Ala Asp Leu Leu
 370 375 380

Leu Val Glu Gly Asn Pro Leu Glu Asp Ile Asp Ala Thr Leu Asn Ile
 385 390 395 400

Arg Gly Val Trp Arg Asp Gly Asn Leu Cys Ser Thr Tyr Val Glu Lys
 405 410 415

Leu Gly Ala Gly Val Glu Pro Leu Leu Ser
 420 425

<210> SEQ ID NO 23

<211> LENGTH: 714

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 23

atgggctccg gatcgctcga tagcacccagtttccaga gctgggactt gtggcagaag	60
atgacttttg tactggcttg cgaaattgtc gtcaccatct tcgttgcct gctcaaactc	120
tggtatgaca agaacaaggatcgcaagtac agcaagggtcg acaagggcaa acgggcgtcg	180
acgcccggaaa tgctcgaggc gcagccagta acccagggttc aagaagacac caaagatgag	240
attccctttg gtatccgcgc aatccaaagc ggcatcgagg ttgatggcgt ctggatctcg	300
cgtaccaaca ctcctgttgg cagtagccgt gcttccatca tgagcgaaca gcttccccgc	360
aacttcaaca actcccagct cgagctgccc cagccagtcg cccagggttc aagccgcaac	420
agtcgcgcgc ctccctagctc gtttgcgtt gccgtctccg ccgagccctt tccaaagctac	480
gactccgcgc catcttcgccc tggccgcggg cacaaccatg agggccctcg ctgcagcaac	540
tgcaaccacc acgtctcccg caacgctcgcc gccctcagcg ccctcgagtc tcccaactct	600
acccgcaact ctgctgctcc ttgcctctt cttcaagcca aacacagcca gtctgcaagc	660
tcctcgagcc gacgcacgag tgacgagtcg gactacatgg ccattggca agac	714

<210> SEQ ID NO 24

<211> LENGTH: 238

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 24

Met Gly Ser Gly Ser Ser Asp Ser Thr Glu Phe Phe Gln Ser Trp Asp
 1 5 10 15

Leu Trp Gln Lys Met Thr Phe Val Leu Ala Cys Gly Ile Val Val Thr
 20 25 30

Ile Phe Val Gly Leu Leu Lys Leu Trp Tyr Asp Lys Asn Lys Val Arg
 35 40 45

Lys Tyr Ser Lys Val Asp Lys Gly Lys Arg Ala Ser Thr Pro Glu Met
 50 55 60

Leu Glu Ala Gln Pro Val Thr Gln Val Gln Glu Asp Thr Lys Asp Glu
 65 70 75 80

Ile Pro Phe Gly Ile Arg Ala Ile Gln Ser Gly Ile Glu Val Asp Gly
 85 90 95

Val Trp Ile Ser Arg Thr Asn Thr Pro Val Gly Ser Ser Arg Ala Ser
 100 105 110

Ile Met Ser Glu Gln Leu Pro Arg Asn Phe Asn Asn Ser Gln Leu Glu
 115 120 125

Leu Pro Gln Pro Val Ala Gln Gly Ser Ser Arg Asn Ser Ser Arg Ala
 130 135 140

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Pro Ser Ser Phe Asp Arg Ala Val Ser Ala Glu Pro Leu Pro Ser Tyr
 145 150 155 160

Asp Ser Arg Ala Ser Ser Pro Gly Arg Gly His Asn His Glu Gly Pro
 165 170 175

Arg Cys Ser Asn Cys Asn His His Val Ser Arg Asn Ala Ala Ala Leu
 180 185 190

Ser Ala Leu Glu Ser Pro Asn Ser Thr Arg Asn Ser Ala Ala Pro Ser
 195 200 205

Pro Pro Leu Gln Ala Lys His Ser Gln Ser Ala Ser Ser Ser Arg
 210 215 220

Arg Thr Ser Asp Glu Ser Asp Tyr Met Ala Ile Gly Gln Asp
 225 230 235

<210> SEQ ID NO 25

<211> LENGTH: 451

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 25

atgtgegtgg atgtgtgggt atggaaatgg tcgggtggccg atggtgtcgt tcgcgtggtg	60
aagctccaac gcggcgccca tggacgcccgg gaactagccg tcgcctcgac tggccggacc	120
ctgggttatga cgcgcgtggcc ccatgccccat cagatgcctc aagaggagcc cggagacggc	180
agcacccacg aaacccaatc ccaaacgcga atgccgcccc acaaccagag cagccagagc	240
aagcgcgaagc acaaatcaaca cagccgtcac aaagagggtgg cggacgaggt ggcaggggac	300
gagggcaagg gcaagggcga gggcgagggc gagggcgagg ggggcaagca gacagtgaaa	360
ggccttcgca accaaatgct gccgcctctcg aatttgcgtt ttcatctgtta caagaagcag	420
cgcacatcgagg aggaagacgt ggacgtgggg g	451

<210> SEQ ID NO 26

<211> LENGTH: 150

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 26

Met Cys Val Asp Val Trp Val Trp Glu Trp Ser Val Ala Asp Gly Val
 1 5 10 15

Val Arg Val Val Lys Leu Gln Arg Gly Gly His Gly Arg Pro Glu Leu
 20 25 30

Ala Val Ala Ser Thr Gly Arg Thr Leu Gly Met Thr Arg Trp Pro His
 35 40 45

Ala His Gln Met Pro Gln Glu Glu Pro Gly Asp Gly Ser Thr His Glu
 50 55 60

Thr Glu Ser Gln Thr Arg Met Pro Pro His Asn Gln Ser Ser Gln Ser
 65 70 75 80

Lys Arg Lys His Asn Gln His Ser Arg His Lys Glu Val Ala Asp Glu
 85 90 95

Val Ala Gly Asp Glu Gly Lys Gly Lys Glu Gly Glu Gly Glu Gly
 100 105 110

Glu Gly Gly Lys Gln Thr Val Lys Gly Leu Arg Asn Gln Met Leu Pro
 115 120 125

Leu Ser Asn Leu Cys Leu His Leu Tyr Lys Lys Gln Arg Ile Glu Glu
 130 135 140

Glu Asp Val Asp Val Gly
 145 150

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<210> SEQ ID NO 27
<211> LENGTH: 1023
<212> TYPE: DNA
<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 27

atggccgcca	ccactacaaa	tcatggcact	aacacgcctc	ctagcacaat	gacatccgca	60
ccacaatac	agcccaagtt	cctgccaaac	aggcatgacc	taggcattcg	cgcagtccgc	120
ttcageggcg	gccagccaa	agccggcgctc	gacgcccgcgc	ccatggccct	catcgaaaat	180
ggcctcatca	agcaattaga	agaagatcta	gaattctccg	tacacctacga	cggccaagtg	240
cacaactaca	ccgagctcca	gccctccgac	gacccagact	accggggcat	gaagegc(ccc	300
aagttcgctt	cgccgcgtcac	aaagcaagtc	tctgaccaag	tctacgagca	cggccaagtgc	360
ggcaagctgg	tcctcaccct	cgggggcgac	cactccatcg	ccattggcac	tgttccggc	420
accgcaaagg	ctattcgca	gccccgtggc	aaggacatgg	ccgtcatctg	ggtcgatgcg	480
catgctgata	ttaatacgcc	cgagacgac	gattcggca	acatccacgg	catgcccgtg	540
tctttcttga	cggggcttggc	gacccgggg	cggggaaatgg	tgtttggctg	gattaaagag	600
gatcagagga	ttagcacgaa	gaagcttagta	tacattggat	tgagggacat	tgatagtgga	660
gagaagaaga	ttctgaggca	gcaacggatc	aaggcggttta	gcatgcata	tattgacagg	720
cacggatttg	gcaaaatcat	ggacatggcg	ctgggttgg	tccggacgc	cacgcccattc	780
catctctctt	tccgtgtca	cgctctcgac	ccatgtggg	ccgttgcac	cggtacgcct	840
gttcgeggcg	gcctgacgct	gcggggggc	gacttcatcg	ccgagtgcgt	tgccgagact	900
ggtcagtc	tgccttgg	tctggtcag	gtgaatctt	gccttgcac	cgaggggtgct	960
ggcgacacgg	tccggctgg	tgtttcgatt	gtggggcg	cgcttggta	cacgttttg	1020
tag						1023

<210> SEQ ID NO 28
<211> LENGTH: 340
<212> TYPE: PRT
<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 28

Met	Ala	Ala	Thr	Thr	Thr	Asn	His	Gly	Thr	Asn	Thr	Pro	Pro	Ser	Thr
1						5			10			15			
Met	Thr	Ser	Ala	Pro	Thr	Ile	Gln	Pro	Lys	Phe	Leu	Pro	Asn	Arg	His
	20					25			30						
Asp	Leu	Gly	Ile	Val	Ala	Val	Gly	Phe	Ser	Gly	Gly	Gln	Pro	Lys	Ala
	35					40			45						
Gly	Val	Asp	Ala	Ala	Pro	Met	Ala	Leu	Ile	Glu	Asn	Gly	Leu	Ile	Lys
	50					55			60						
Gln	Leu	Glu	Glu	Asp	Leu	Glu	Phe	Ser	Val	Thr	Tyr	Asp	Gly	Gln	Val
	65					70			75			80			
His	Asn	Tyr	Thr	Glu	Leu	Gln	Pro	Ser	Asp	Asp	Pro	Asp	Tyr	Arg	Gly
	85					90			95						
Met	Lys	Arg	Pro	Lys	Phe	Ala	Ser	Ala	Val	Thr	Lys	Gln	Val	Ser	Asp
	100					105			110						
Gln	Val	Tyr	Glu	His	Ala	Lys	Ser	Gly	Lys	Leu	Val	Leu	Thr	Leu	Gly
	115					120			125						
Gly	Asp	His	Ser	Ile	Ala	Ile	Gly	Thr	Val	Ser	Gly	Thr	Ala	Lys	Ala
	130					135			140						
Ile	Arg	Glu	Arg	Leu	Gly	Lys	Asp	Met	Ala	Val	Ile	Trp	Val	Asp	Ala

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145	150	155	160
His Ala Asp Ile Asn Thr Pro Glu Thr Ser Asp Ser Gly Asn Ile His			
	165	170	175
Gly Met Pro Val Ser Phe Leu Thr Gly Leu Ala Thr Glu Arg Glu			
	180	185	190
Asp Val Phe Gly Trp Ile Lys Glu Asp Gln Arg Ile Ser Thr Lys Lys			
	195	200	205
Leu Val Tyr Ile Gly Leu Arg Asp Ile Asp Ser Gly Glu Lys Lys Ile			
	210	215	220
Leu Arg Gln His Gly Ile Lys Ala Phe Ser Met His Asp Ile Asp Arg			
	225	230	235
			240
His Gly Ile Gly Lys Ile Met Asp Met Ala Leu Gly Trp Ile Gly Ser			
	245	250	255
Asp Thr Pro Ile His Leu Ser Phe Asp Val Asp Ala Leu Asp Pro Met			
	260	265	270
Trp Ala Pro Ser Thr Gly Thr Pro Val Arg Gly Gly Leu Thr Leu Arg			
	275	280	285
Glu Gly Asp Phe Ile Ala Glu Cys Val Ala Glu Thr Gly Gln Leu Ile			
	290	295	300
Ala Leu Asp Leu Val Glu Val Asn Pro Ser Leu Asp Ala Glu Gly Ala			
	305	310	315
			320
Gly Asp Thr Val Arg Ala Gly Val Ser Ile Val Arg Cys Ala Leu Gly			
	325	330	335
Asp Thr Leu Leu			
	340		

<210> SEQ ID NO 29

<211> LENGTH: 1371

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 29

atgtacagga cactcgctct cgcttccctc tcgctttcg gagccgcccc cgctcagcag	60
gttggcaaag agacaacgga gacacacccc aagatgacat ggcagacttg cactggcacc	120
ggtggaaaga gctgcaccaa taaggcagggt tccatcgtc tcgactccaa ctggcgatgg	180
tcccacgtca ccagcggata caccaactgc ttgcacggca actcttggaa cacgaccgct	240
tgcctgtatg gcagcacttg caccaagaac tgccatcg acggtgccga ttactctggc	300
acttaacggca tcaccaccag cagcaatgtc tgcacttc acatggctct	360
tactctgccca acattggttc acgttacctac ctcatggaga gtgacaccaa gtaccaaattg	420
ttcaatctca tcggcaagga gttcaccttc gatgtcgatg tctccaagct gccttgcgggt	480
ctgaacgggt ctctctactt tggttggatgc gcccggacgt gtggcatgaa caaggggcaac	540
aacaaggccg gtgccaagta cggaaacccgga tactgcgact cccagtgc ctcacgacatc	600
aagtttatca acgggttagc caacgttagag ggcttggaaacc cgtccgacaa tgaccccaac	660
gccggcgctg gtaaggattgg tgcttgcgtc cccgaaatgg atatctggaa ggcacactcc	720
atctctactg cctacactcc ccattccctgc aaggcactg gtcttcagga gtgcactgac	780
gaggtcagct gcgggtatgg cgacaacccgt tacggcggtt tctgcgacaa ggacgggtgc	840
gatttcaaca gtcacccgtat ggggttccgt gacttctacg gtccaggcat gaccctcgat	900
accaccaaga agatgactgt cgtcaactcg ttcctcggtt ccgggttccag cctctcgag	960
atcaaggcgct tctacatcca gggaggaacc gtcttcaaga actccgactc cgccgtcgaa	1020

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ggcgtcaactg gtaactccat cactgaggaa ttctgtgacc agcaaaagac cgtttcggt 1080
gacacatctt ctttcaagac tcttggtgga ctgtatgaga tgggtgcctc gcttgctgc 1140
ggtcacgtcc ttgtcatgtc cctttggac gaccatgcgg tcaacatgct ttggctcgac 1200
tccacacctacc ctaccgacgc tgaccaggag aagcctggta tcgcccgtgg tacctgcgct 1260
accgactctg gcaagccccga ggacgtcgag gccaactcgc cccgacgcgac tgtcatcttc 1320
tccaacatca agttcggtcc catcggtcc acctttccg cacccgcata a 1371

```

<210> SEQ ID NO 30

<211> LENGTH: 456

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 30

```

Met Tyr Arg Thr Leu Ala Leu Ala Ser Leu Ser Leu Phe Gly Ala Ala
1 5 10 15

```

```

Arg Ala Gln Gln Val Gly Lys Glu Thr Thr Glu Thr His Pro Lys Met
20 25 30

```

```

Thr Trp Gln Thr Cys Thr Gly Thr Gly Lys Ser Cys Thr Asn Lys
35 40 45

```

```

Gln Gly Ser Ile Val Leu Asp Ser Asn Trp Arg Trp Ser His Val Thr
50 55 60

```

```

Ser Gly Tyr Thr Asn Cys Phe Asp Gly Asn Ser Trp Asn Thr Thr Ala
65 70 75 80

```

```

Cys Pro Asp Gly Ser Thr Cys Thr Lys Asn Cys Ala Ile Asp Gly Ala
85 90 95

```

```

Asp Tyr Ser Gly Thr Tyr Gly Ile Thr Thr Ser Ser Asn Ala Leu Thr
100 105 110

```

```

Leu Lys Phe Val Thr Lys Gly Ser Tyr Ser Ala Asn Ile Gly Ser Arg
115 120 125

```

```

Thr Tyr Leu Met Glu Ser Asp Thr Lys Tyr Gln Met Phe Asn Leu Ile
130 135 140

```

```

Gly Lys Glu Phe Thr Phe Asp Val Asp Val Ser Lys Leu Pro Cys Gly
145 150 155 160

```

```

Leu Asn Gly Ala Leu Tyr Phe Val Glu Met Ala Ala Asp Gly Gly Met
165 170 175

```

```

Asn Lys Gly Asn Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys
180 185 190

```

```

Asp Ser Gln Cys Pro His Asp Ile Lys Phe Ile Asn Gly Val Ala Asn
195 200 205

```

```

Val Glu Gly Trp Asn Pro Ser Asp Asn Asp Pro Asn Ala Gly Ala Gly
210 215 220

```

```

Lys Ile Gly Ala Cys Cys Pro Glu Met Asp Ile Trp Glu Ala Asn Ser
225 230 235 240

```

```

Ile Ser Thr Ala Tyr Thr Pro His Pro Cys Lys Gly Thr Gly Leu Gln
245 250 255

```

```

Glu Cys Thr Asp Glu Val Ser Cys Gly Asp Gly Asp Asn Arg Tyr Gly
260 265 270

```

```

Gly Ile Cys Asp Lys Asp Gly Cys Asp Phe Asn Ser Tyr Arg Met Gly
275 280 285

```

```

Val Arg Asp Phe Tyr Gly Pro Gly Met Thr Leu Asp Thr Thr Lys Lys
290 295 300

```

```

Met Thr Val Val Thr Gln Phe Leu Gly Ser Gly Ser Ser Leu Ser Glu
305 310 315 320

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Ile	Lys	Arg	Phe	Tyr	Ile	Gln	Gly	Gly	Thr	Val	Phe	Lys	Asn	Ser	Asp
325						330				335					
Ser	Ala	Val	Glu	Gly	Val	Thr	Gly	Asn	Ser	Ile	Thr	Glu	Glu	Phe	Cys
340						345					350				
Asp	Gln	Gln	Lys	Thr	Val	Phe	Gly	Asp	Thr	Ser	Ser	Phe	Lys	Thr	Leu
355						360				365					
Gly	Gly	Leu	Asp	Glu	Met	Gly	Ala	Ser	Leu	Ala	Arg	Gly	His	Val	Leu
370						375				380					
Val	Met	Ser	Leu	Trp	Asp	Asp	His	Ala	Val	Asn	Met	Leu	Trp	Leu	Asp
385						390				395			400		
Ser	Thr	Tyr	Pro	Thr	Asp	Ala	Asp	Pro	Glu	Lys	Pro	Gly	Ile	Ala	Arg
405							410				415				
Gly	Thr	Cys	Ala	Thr	Asp	Ser	Gly	Lys	Pro	Glu	Asp	Val	Glu	Ala	Asn
420							425				430				
Ser	Pro	Asp	Ala	Thr	Val	Ile	Phe	Ser	Asn	Ile	Lys	Phe	Gly	Pro	Ile
435							440				445				
Gly	Ser	Thr	Phe	Ser	Ala	Pro	Ala								
450						455									

<210> SEQ ID NO 31

<211> LENGTH: 1203

<212> TYPE: DNA

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 31

atgcgtctcca	acacctttct	cactgctcg	cttgcagtag	gggtggctca	ggccctgcct	60
caaggcaca	gtgtctcgag	gactacatct	accggccgtg	caacgaccac	tgcggcatca	120
gcaactggaa	acccttcgc	tggcaaggat	ttctatgcca	accctatacta	ctcgccgag	180
gtttacaccc	tagccatgcc	ctcgcttgct	gggtctctga	agcccgctgc	ttctgcccgt	240
gccaaggatcg	gttcattcgat	atggatggac	acaatggcca	agggtccccac	catggacacg	300
tatctggcag	acatcaaagc	caagaatgcc	gcaggtgcaa	agctgtatggg	tactttgc	360
gtctacgacc	tgcccgaccg	cgactgcgt	gcccttgcct	ccaacggcga	gctcaagatc	420
gacgacggtg	gtgttagagaa	gtacaagacc	cagtacatcg	acaagattgc	cgcttattatt	480
aaggcgatcc	ctgacattaa	gatcaaccc	gccatttgcgc	ccgactcggtt	ggccaacatg	540
gtcaccaaca	tgggcttaca	aaagtgcgt	cgccggcgctc	cctactacaa	agagttacc	600
gctgtacgtct	tcaagacgtct	caatttcccc	aacgtcgaca	tgtacccgt	cggtggccac	660
gctggctggc	ttggctggga	cgccaacatt	ggtccagccg	caaaaactcta	cgccgaagtc	720
tacaaggccg	ctggctcgcc	ccggccgttc	cgtggatcg	taccaacgt	cagcaactac	780
aacgccttcc	gcatggcac	ttggccctgct	atcacccaa	gaaacaagaa	ctgcgacgaa	840
gagcgttca	tcgacgtttt	cgtcccttct	ctccgcgcgg	aaaggctccc	tgcccaacttc	900
atcgtegaca	ctggacgttag	cggtaagcag	cctactgacc	agcaggccctg	gggagactgg	960
tgcaacgttt	cggggtctgg	cttggatatt	cgtccctacta	ccaacaccaa	caatgcgttt	1020
gtcgatgctt	ttgtctgggt	caaggcttgt	ggcgagtctg	atggtacttc	tgaccaatct	1080
gctgctcgct	acgacggctt	ctggggcaag	gcctccgtt	tgaaggctgc	gccccgaggct	1140
ggtacttggt	tccaggcata	ctttgagatg	ttgttaaaga	acgccaaccc	cgctttgca	1200
taa						1203

<210> SEQ ID NO 32

<211> LENGTH: 400

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<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 32

Met Leu Ser Asn Leu Leu Leu Thr Ala Ala Leu Ala Val Gly Val Ala
 1 5 10 15

 Gln Ala Leu Pro Gln Ala Thr Ser Val Ser Arg Thr Thr Ser Thr Ala
 20 25 30

 Arg Ala Thr Thr Thr Ala Pro Ser Ala Thr Gly Asn Pro Phe Ala Gly
 35 40 45

 Lys Asp Phe Tyr Ala Asn Pro Tyr Tyr Ser Ser Glu Val Tyr Thr Leu
 50 55 60

 Ala Met Pro Ser Leu Ala Ala Ser Leu Lys Pro Ala Ala Ser Ala Val
 65 70 75 80

 Ala Lys Val Gly Ser Phe Val Trp Met Asp Thr Met Ala Lys Val Pro
 85 90 95

 Thr Met Asp Thr Tyr Leu Ala Asp Ile Lys Ala Lys Asn Ala Ala Gly
 100 105 110

 Ala Lys Leu Met Gly Thr Phe Val Val Tyr Asp Leu Pro Asp Arg Asp
 115 120 125

 Cys Ala Ala Leu Ala Ser Asn Gly Glu Leu Lys Ile Asp Asp Gly Gly
 130 135 140

 Val Glu Lys Tyr Lys Thr Gln Tyr Ile Asp Lys Ile Ala Ala Ile Ile
 145 150 155 160

 Lys Ala Tyr Pro Asp Ile Lys Ile Asn Leu Ala Ile Glu Pro Asp Ser
 165 170 175

 Leu Ala Asn Met Val Thr Asn Met Gly Val Gln Lys Cys Ser Arg Ala
 180 185 190

 Ala Pro Tyr Tyr Lys Glu Leu Thr Ala Tyr Ala Leu Lys Thr Leu Asn
 195 200 205

 Phe Pro Asn Val Asp Met Tyr Leu Asp Gly Gly His Ala Gly Trp Leu
 210 215 220

 Gly Trp Asp Ala Asn Ile Gly Pro Ala Ala Lys Leu Tyr Ala Glu Val
 225 230 235 240

 Tyr Lys Ala Ala Gly Ser Pro Arg Ala Val Arg Gly Ile Val Thr Asn
 245 250 255

 Val Ser Asn Tyr Asn Ala Phe Arg Ile Gly Thr Cys Pro Ala Ile Thr
 260 265 270

 Gln Gly Asn Lys Asn Cys Asp Glu Arg Phe Ile Asp Ala Phe Ala
 275 280 285

 Pro Leu Leu Arg Ala Glu Gly Phe Pro Ala His Phe Ile Val Asp Thr
 290 295 300

 Gly Arg Ser Gly Lys Gln Pro Thr Asp Gln Gln Ala Trp Gly Asp Trp
 305 310 315 320

 Cys Asn Val Ser Gly Ala Gly Phe Gly Ile Arg Pro Thr Thr Asn Thr
 325 330 335

 Asn Asn Ala Leu Val Asp Ala Phe Val Trp Val Lys Pro Gly Gly Glu
 340 345 350

 Ser Asp Gly Thr Ser Asp Gln Ser Ala Ala Arg Tyr Asp Gly Phe Cys
 355 360 365

 Gly Lys Ala Ser Ala Leu Lys Pro Ala Pro Glu Ala Gly Thr Trp Phe
 370 375 380

 Gln Ala Tyr Phe Glu Met Leu Leu Lys Asn Ala Asn Pro Ala Leu Ala
 385 390 395 400

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<210> SEQ ID NO 33
<211> LENGTH: 2667
<212> TYPE: DNA
<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 33

atgaagacaa	tttcttttgt	tcaaggcggt	tegctgtat	ccactcttt	cgtctcttc	60
getcttgcgc	aggagaagtt	tacccaccaa	ggtagccggaa	tttagttctg	gcccaggta	120
gtcagtgact	cccgactgc	aggaggcttc	gagtggggct	gggtattgcc	agcagagccc	180
actggagcca	acgacaaata	catacggtac	attaaaggtt	cgttggaaac	gaacagacag	240
ggatggtccg	gtgtcagcca	cgcgtggc	atggctaact	ctctttgtc	cgttgcattgg	300
ccggaaaactg	atgtgtcaa	gaccaagttt	gtctggcag	gtggctatata	tgctctgaa	360
gactacactg	gcaacgcac	tttgagccag	atcttcact	cagtaccga	cacacacttc	420
gagatcgatgt	accgatgcga	gcactgctgg	gtctggaaatc	agggtggtgc	tgaaggctcc	480
caactcccc	ccagcgaagt	caatgttatac	ggctggggcc	agcataacaa	aatctacgac	540
ggcacttggg	tcttccacaa	caaggacag	tccctgtttt	gtgctctac	ggtggatgca	600
aggaacgcga	agtactccga	ctatgtcaaa	ctggcaggag	gccagccatc	ttgtgcacct	660
acaccaacct	tgtccggcca	gcccgtaccc	acacccactc	ccactgcacc	ggtaaagtgc	720
accggatccc	cagcccccttc	aggttccctt	gactacatcg	tcattggtgg	ttgtgctgaa	780
ggtatcccc	ttggcgacag	gctttccgag	tctggcaaga	gggttctcat	gctcgagaag	840
ggcccgccgt	ccctcgctcg	ttttggcgga	aagatggggcc	ctgaatgggc	taccaccaac	900
aatttgactc	ggtgcacat	ccctggcttc	tgcaaccaga	tctgggttga	ctctgcaggt	960
tttgcttgca	ccgatatcga	ccaaatgggt	ggctgtgtcc	ttgggtggagg	tactggcgcc	1020
aatgtcgcc	tttgggtggaa	ggccgttagac	atcgatttcg	actaccattt	ccccgctggc	1080
tggaaatcag	cggaacgtgaa	gggcgcgatc	gaccgtgtgt	tcaagegcata	ccctggact	1140
gataccctt	ccgtggacgg	caagcgttac	aagcaggaaag	gctttgatgt	cctatccggt	1200
gcgccttggtg	cgatggctg	gaagagcgatc	gtcgcgaacg	accaacagaa	ccagaagaat	1260
cgcacatact	ctcaactctcc	gttcatgtat	gacaacgggt	aaaggcaagg	acctctcggt	1320
acttacatgg	tttctgcgtt	ggaaaggaag	aacttcaagc	tctggacgaa	caccatggct	1380
cgacgcacatcg	tccgcactgg	cggaacggct	accgggtttt	agcttgagag	cggtgtcggt	1440
ggtactgggtt	actcgccgtac	cgtcaaccc	aaccctggag	gcccgtttat	tgtctccgggt	1500
ggagctttcg	gatcgatcaa	ggttcttcc	cgcagcggca	ttggacaaaa	ggatcagctg	1560
aacatcgatcg	agaacagcgc	tctcgatggc	tgcacaatga	ttggagagtc	tgactggatt	1620
accctcccc	tcggccaaaa	cttgaacgcac	cacgtcaaca	ccgatcttgt	tatcaggcac	1680
cccaacatct	cttcctacaa	cttttacgag	gcgtgggatg	cccccatcga	ggctgacaaa	1740
gacctgtacc	ttggcaagcg	tttgcgttac	cttgcacccaa	catggcccc	1800	
cttgcttggg	aagtgattac	tggaaagtgc	ggcattgacc	gatcgatcca	gtggactgct	1860
cgtgttgaag	gccccggcgc	caacgatact	caccacctca	ccatcagcca	gtacctcggt	1920
cacggctcta	tttcgcgtgg	tgcgctttcc	atcaacgggt	ctctcaacgt	gtatgtcagc	1980
aaatcacctt	acctacagaa	cgaggccgac	actgggtgtgg	ttgtcgcagg	tatcaagagc	2040
atgatgaagg	ccatccagaa	gaacccagcc	atcgagttcc	aagtaccggc	tgccaatatg	2100
acagttgagg	catacggtgc	cagccctcccc	aagaccccaag	ctgcccgtcg	cgccaaccac	2160

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tggatcggtta	ccgccaagat	cggAACCGAC	agcggTCTCA	cgggtggAAC	ctctgtggTG	2220
gacctgaaca	ctcagggtgtA	tggAACGCG	aacatCCACG	tagtcgacGC	ttcgcttC	2280
cctggtaaaa	ttttcaccaa	ccctacatCC	tacatcatCG	tactcgcaga	acatGCCGT	2340
getaagattc	tcgcacttag	tgcaagcagt	ggaggtggta	agccttcgtc	gtccgcttg	2400
tegtccgcag	tctccgctaa	accactacc	tegaaggcac	caactgagTC	gtcaaccgta	2460
tcgggtggAGC	gtccatcgac	accagccaag	tcttcggcta	agtcgactac	tatcaagaca	2520
tctgcagcac	cagcacctac	tcctaccagg	gtgtcgaagg	cotggaaacg	atgcgggtgt	2580
aaaggctaca	ctggcccaac	agcttgtgtc	agtggcaca	agtgcgcagt	gagcaatgag	2640
tactactctc	agtgeatccc	taactaa				2667

<210> SEQ ID NO 34

<211> LENGTH: 888

<212> TYPE: PRT

<213> ORGANISM: Alternaria brassicicola

<400> SEQUENCE: 34

Met	Lys	Thr	Thr	Ser	Phe	Val	Gln	Ala	Ala	Ser	Leu	Leu	Ser	Thr	Leu
1					5				10					15	

Phe	Ala	Pro	Leu	Ala	Leu	Ala	Gln	Glu	Lys	Phe	Thr	His	Glu	Gly	Thr
			20					25					30		

Gly	Ile	Glu	Phe	Trp	Arg	Gln	Val	Val	Ser	Asp	Ser	Gln	Thr	Ala	Gly
	35					40						45			

Gly	Phe	Glu	Trp	Gly	Trp	Val	Leu	Pro	Ala	Glu	Pro	Thr	Gly	Ala	Asn
	50					55				60					

Asp	Glu	Tyr	Ile	Gly	Tyr	Ile	Lys	Gly	Ser	Leu	Glu	Ala	Asn	Arg	Gln
65						70			75					80	

Gly	Trp	Ser	Gly	Val	Ser	His	Ala	Gly	Gly	Met	Ala	Asn	Ser	Leu	Leu
	85					90						95			

Leu	Val	Ala	Trp	Pro	Glu	Thr	Asp	Ala	Val	Lys	Thr	Lys	Phe	Val	Trp
	100					105				110					

Ala	Gly	Gly	Tyr	Ile	Ala	Pro	Glu	Asp	Tyr	Thr	Gly	Asn	Ala	Thr	Leu
	115					120				125					

Ser	Gln	Ile	Phe	His	Ser	Val	Thr	Asp	Thr	His	Phe	Glu	Ile	Val	Tyr
	130					135				140					

Arg	Cys	Glu	His	Cys	Trp	Val	Trp	Asn	Gln	Gly	Gly	Ala	Glu	Gly	Ser
145						150			155				160		

Gln	Leu	Pro	Thr	Ser	Glu	Val	Asn	Val	Ile	Gly	Trp	Ala	Gln	His	Asn
	165					170				175					

Lys	Ile	Tyr	Asp	Gly	Thr	Trp	Val	Phe	His	Asn	Lys	Gly	Gln	Ser	Leu
	180					185				190					

Phe	Gly	Ala	Pro	Thr	Val	Asp	Ala	Arg	Asn	Ala	Lys	Tyr	Ser	Asp	Tyr
	195					200				205					

Val	Lys	Leu	Ala	Gly	Gly	Gln	Pro	Ser	Gly	Ala	Pro	Thr	Pro	Thr	Leu
	210					215				220					

Ser	Gly	Gln	Pro	Ser	Ala	Thr	Pro	Thr	Pro	Ala	Pro	Val	Lys	Cys	
225						230			235				240		

Thr	Gly	Ser	Pro	Ala	Pro	Ser	Gly	Ser	Phe	Asp	Tyr	Ile	Val	Ile	Gly
	245					250				255					

Gly	Gly	Ala	Gly	Gly	Ile	Pro	Met	Ala	Asp	Arg	Leu	Ser	Glu	Ser	Gly
	260				265						270				

Lys	Ser	Val	Leu	Met	Leu	Glu	Lys	Gly	Pro	Pro	Ser	Leu	Ala	Arg	Phe
	275				280						285				

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Gly Gly Lys Met Gly Pro Glu Trp Ala Thr Thr Asn Asn Leu Thr Arg
290 295 300

Phe Asp Ile Pro Gly Leu Cys Asn Gln Ile Trp Val Asp Ser Ala Gly
305 310 315 320

Val Ala Cys Thr Asp Ile Asp Gln Met Ala Gly Cys Val Leu Gly Gly
325 330 335

Gly Thr Ala Val Asn Ala Ala Leu Trp Trp Lys Pro Val Asp Ile Asp
340 345 350

Phe Asp Tyr Gln Phe Pro Ala Gly Trp Lys Ser Ala Asp Val Lys Gly
355 360 365

Ala Ile Asp Arg Val Phe Lys Arg Ile Pro Gly Thr Asp Thr Pro Ser
370 375 380

Val Asp Gly Lys Arg Tyr Lys Gln Glu Gly Phe Asp Val Leu Ser Gly
385 390 395 400

Ala Leu Gly Ala Asp Gly Trp Lys Ser Val Val Ala Asn Asp Gln Gln
405 410 415

Asn Gln Lys Asn Arg Thr Tyr Ser His Ser Pro Phe Met Tyr Asp Asn
420 425 430

Gly Gln Arg Gln Gly Pro Leu Gly Thr Tyr Met Val Ser Ala Leu Glu
435 440 445

Arg Lys Asn Phe Lys Leu Trp Thr Asn Thr Met Ala Arg Arg Ile Val
450 455 460

Arg Thr Gly Gly Thr Ala Thr Gly Val Glu Leu Glu Ser Gly Val Gly
465 470 475 480

Gly Thr Gly Tyr Cys Gly Thr Val Asn Leu Asn Pro Gly Gly Arg Val
485 490 495

Ile Val Ser Gly Gly Ala Phe Gly Ser Ser Lys Val Leu Phe Arg Ser
500 505 510

Gly Ile Gly Pro Lys Asp Gln Leu Asn Ile Val Lys Asn Ser Ala Leu
515 520 525

Asp Gly Ser Thr Met Ile Gly Glu Ser Asp Trp Ile Asn Leu Pro Val
530 535 540

Gly Gln Asn Leu Asn Asp His Val Asn Thr Asp Leu Val Ile Arg His
545 550 555 560

Pro Asn Ile Ser Ser Tyr Asn Phe Tyr Glu Ala Trp Asp Ala Pro Ile
565 570 575

Glu Ala Asp Lys Asp Leu Tyr Leu Gly Lys Arg Ser Gly Ile Leu Ala
580 585 590

Gln Ser Ala Pro Asn Ile Gly Pro Leu Ala Trp Glu Val Ile Thr Gly
595 600 605

Ser Asp Gly Ile Asp Arg Ser Ile Gln Trp Thr Ala Arg Val Glu Gly
610 615 620

Pro Gly Ala Asn Asp Thr His His Leu Thr Ile Ser Gln Tyr Leu Gly
625 630 635 640

His Gly Ser Thr Ser Arg Gly Ala Leu Ser Ile Asn Gly Ala Leu Asn
645 650 655

Val Tyr Val Ser Lys Ser Pro Tyr Leu Gln Asn Glu Ala Asp Thr Gly
660 665 670

Val Val Val Ala Gly Ile Lys Ser Met Met Lys Ala Ile Gln Lys Asn
675 680 685

Pro Ala Ile Glu Phe Gln Val Pro Pro Ala Asn Met Thr Val Glu Ala
690 695 700

Tyr Val Ala Ser Leu Pro Lys Thr Pro Ala Ala Arg Arg Ala Asn His
705 710 715 720

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Trp Ile Gly Thr Ala Lys Ile Gly Thr Asp Ser Gly Leu Thr Gly Gly
725 730 735

Thr Ser Val Val Asp Leu Asn Thr Gln Val Tyr Gly Thr Gln Asn Ile
740 745 750

His Val Val Asp Ala Ser Leu Phe Pro Gly Gln Ile Phe Thr Asn Pro
755 760 765

Thr Ser Tyr Ile Ile Val Leu Ala Glu His Ala Ala Ala Lys Ile Leu
770 775 780

Ala Leu Ser Ala Ser Ser Gly Gly Lys Pro Ser Ser Ser Ala Leu
785 790 795 800

Ser Ser Ala Val Ser Ala Lys Pro Thr Thr Ser Lys Ala Pro Thr Glu
805 810 815

Ser Ser Thr Val Ser Val Glu Arg Pro Ser Thr Pro Ala Lys Ser Ser
820 825 830

Ala Lys Ser Thr Thr Ile Lys Thr Ser Ala Ala Pro Ala Pro Thr Pro
835 840 845

Thr Arg Val Ser Lys Ala Trp Glu Arg Cys Gly Lys Gly Tyr Thr
850 855 860

Gly Pro Thr Ala Cys Val Ser Gly His Lys Cys Ala Val Ser Asn Glu
865 870 875 880

Tyr Tyr Ser Gln Cys Ile Pro Asn
885

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated oligonucleotide
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Leu Ser Ile Gly Lys Val
1 5

<210> SEQ ID NO 36
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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Gly Leu Ile Val Lys Ser
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated oligonucleotide
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Ser Lys Gly Arg Ser Leu Ile Gly Lys
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<210> SEQ ID NO 38
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated oligonucleotide

-continued

<400> SEQUENCE: 38

Ser Leu Ile Gly Lys Val
1 5

What is claimed is:

1. A substantially purified polypeptide comprising the 10 amino acid sequence set forth in SEQ ID NO:14.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,972,611 B2
APPLICATION NO. : 12/973100
DATED : July 5, 2011
INVENTOR(S) : Hirohito Kita

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page, Item (75), Inventors, after Christopher Lawrence, please delete "Blackburg" and insert --Blacksburg-- therefor;

Title Page, Item (57), Abstract, before "methods for assessing" please delete "fungal polypeptides,";

Column 1, lines 22-25, please delete "This invention was made with government support under AI049235 awarded by the National Institute of Allergy and Infectious Disease. The government has certain rights in the invention." and insert --This invention was made with government support under AI049235 awarded by the National Institutes of Health. The government has certain rights in the invention.-- therefor.

Signed and Sealed this
Twenty-seventh Day of March, 2012



David J. Kappos
Director of the United States Patent and Trademark Office